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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231
Box: Patent Application
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March 10, 1997

Transmitted herewith for filing are the specification and claims of the patent application of:

1-00 2-00 3-00 4-00 5-00

David Baltimore, Genhong Cheng, Aileen Cleary, Seth Lederman and Zheng-sheng Ye **for Inventor(s)**

TRUNCATED CRAF1 INHIBITS CD40 SIGNALING
Title of Invention

Also enclosed are:

X 11 **sheet(s) of** ____ **informal** X **formal drawings.**

____ **Oath or declaration of Applicant(s).**

____ **A power of attorney**

____ **An assignment of the invention to** _____

X **A Preliminary Amendment**

X **A verified statement to establish small entity status under 37 C.F.R. §1.9 and §1.27.**

The filing fee is calculated as follows:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

	NUMBER FILED		NUMBER EXTRA*		RATE SMALL ENTITY	RATE OTHER ENTITY		FEE SMALL ENTITY	FEE OTHER ENTITY
Total Claims	20 -20	=	0	X	$ 11	$ 22	=	$ 0	$
Independent Claims	2 -3	=	0	X	$ 40	$ 80	=	$ 0	$
Multiple Dependent Claims Presented: ____Yes X No					$130	$260	=	$ 0	$
						BASIC FEE		$ 385	$ 770
						TOTAL FEE		$385	$

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Letter of Transmittal
Page 2

__X__ **A check in the amount of $385.00 to cover the filing fee.**

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__X__ **Three copies of this sheet are enclosed.**

_____ **A certified copy of previously filed foreign application No._____ filed in _____ on _____. Applicant(s) hereby claim priority based upon this aforementioned foreign application under 35 U.S.C. §119.**

__X__ **Other (identify)** Express Mail Certificate of Mailing bearing Label No. EI239 299 250US dated March 10, 1997 and one loose set of formal drawings.

Respectfully submitted,

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Dkt. 50659/JPW/JML

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : David Baltimore et al.

Serial No. : Not Yet Known

Filed : Herewith

Title : TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

1185 Avenue of the Americas
New York, N.Y. 10036
March 10, 1997

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

PRELIMINARY AMENDMENT

Please amend the subject application as follows:

In the Claims:

Please cancel claims 21-91 without prejudice.

REMARKS

Applicants hereinabove have canceled claims 21-91 without prejudice. Applicants respectfully request that these amendments be entered.

If a telephone interview would be of assistance in advancing the prosecution of the subject application, applicants' undersigned attorney hereby invites the Examiner to whom the subject application is assigned to telephone him at the number provided.

David Baltimore et al.
U.S. Serial No.: Not Yet Known
Filed: Herewith
Page 2

No fee is deemed necessary in connection with the filing of this Preliminary Amendment. However, if any fee is required, authorization is hereby given to charge the amount any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

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Application
for
United States Letters Patent

To all whom it may concern:

Be it known that David Baltimore, Genhong Cheng, Aileen Cleary, Seth Lederman and Zheng-sheng Ye

have invented certain new and useful improvements in

TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

of which the following is a full, clear and exact description.

TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

This application claims the benefit of U.S. Provisional No. 60/013,199, filed March 11, 1996, the contents of which are hereby incorporated by reference into the present application.

The invention disclosed herein was made with Government support under NIH Grant Nos. RO1-CA55713 and A122346 from the Department of Health and Human Services. Accordingly, the U.S. Government has certain rights in this invention.

Throughout this application, various references are referred to within parentheses. Disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains. Full bibliographic citation for these references may be found in the text and at the end of this application, preceding the sequence listing and the claims.

The following standard abbreviations are used throughout to refer to amino acids:

A	Ala	Alanine	M	Met	Methionine
C	Cys	Cysteine	N	Asn	Asparagine
D	Asp	Aspartic acid	P	Pro	Proline
E	Glu	Glutamic acid	Q	Gln	Glutamine
F	Phe	Phenylalanine	R	Arg	Arginine
G	Gly	Glycine	S	Ser	Serine
H	His	Histidine	T	Thr	Threonine
I	Ile	Isoleucine	V	Val	Valine
K	Lys	Lysine	W	Trp	Tryptophan
L	Leu	Leucine	Y	Tyr	Tyrosine

Background of the Invention

CD40 (1) is a receptor on B cells that interacts with

the helper T cell surface protein CD40L (CD40 ligand, also known as T-BAM, gp39, or TRAP) (2-4). CD40L is found particularly on lymphoid follicle $CD4^+$ T lymphocytes, where it delivers a contact-dependent signal that stimulates B cell survival, growth, and differentiation (2-4). Signaling through CD40 rescues B cells from apoptosis induced by Fas (CD95) or by cross-linking of the immunoglobulin M (IgM) complex (5); it also induces B cells to differentiate and to undergo Ig isotype switching (3) and to express CD80 (B7 or BB-1) (6). The crucial role of CD40L-CD40 interaction is illustrated by humans with defects in CD40L, who manifest a serious immune deficiency syndrome, the X-linked hyper-IgM syndrome (HIGMX-1) characterized by an absence of IgG, IgA, and IgE, elevated IgM, and no lymphoid follicles (7). The essential roles of CD40L and CD40 in the phenotype of HIGMX-1 syndrome has been confirmed by targeted disruption of either CD40L (8) or CD40 (9) in mice. In addition to B cells, CD40 is also expressed by follicular dendritic cells (10), dendritic cells (11), activated macrophages (12), epithelial cells (including thymic epithelium) (13), and a variety of tumor cells.

Stimulation of CD40 causes the tyrosine phosphorylation of multiple substrates including Src family kinases such as $p53\text{-}p56^{lyn}$, activates multiple serine-threonine-specific protein kinases, and induces the phosphorylation of phospholipase C-γ2 and of phosphoinositide-3' kinase (14).

In mice the CD40 cytoplasmic tail is necessary for signaling (15). Proteins which interact with the cytoplasmic tail of CD40 have been described (H.M. Hu, et al., J. Biol. Chem. 269: 30069 (1994); and G. Mosialos, et al., Cell 80:389 (1995)). These proteins are the same as CRAF1.

<u>Summary of the Invention</u>

This invention provides a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof capable of inhibiting CD40-mediated cell activation.

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, comprising providing the cells with an agent capable of inhibiting CD40-mediated intracellular signaling, the agent being present in an amount effective to inhibit activation of the cells.

This invention provides a method of providing a subject with an amount of a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof effective to inhibit activation by CD40 ligand of cells bearing CD40 on the cell surface in the subject, comprising: introducing into CD40-bearing cells of the subject, a nucleic acid sequence encoding the protein under conditions such that the cells express in the subject an activation inhibiting effective amount of the protein.

This invention provides a method of treating a condition characterized by an aberrant or unwanted level of CD40-mediated intracellular signaling, in a subject, comprising providing the subject with a therapeutically effective amount of an agent capable of inhibiting CD40-mediated intracellular signaling in cells bearing CD40 on the cell surface.

This invention provides a nucleic acid molecule encoding a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof capable of inhibiting CD40-mediated

cell activation.

This invention provides a method of identifying an agent capable of inhibiting CD40-mediated intracellular signaling in a cell expressing CD40 on the cell surface, comprising providing the cell with the agent under conditions permitting activation of the cell in the absence of the agent, and determining decreased or absent activation, thereby identifying an agent capable of inhibiting CD40-mediated intracellular signaling in a cell expressing CD40 on the cell surface.

Description of the Figures

Figure 1. Predicted amino acid sequences of mouse (M) and human (H) CRAF1. The full-length mouse sequence is shown and numbered. The human sequence has one more amino acid than that of the mouse (indicated with a dot), but all numbers here refer to the mouse sequence. Dashes indicate positions in the human sequence that are identical to those in the mouse. The C26 clone obtained from the yeast two-hybrid screen contained the COOH-terminal region of CRAF1 starting from the position marked with an arrow.

Figures 2A-D. Potential structural domains of CRAF1. (**A**) Diagrams of three TRAF family members. Percentages of amino acid identity between CRAF1 and either TRAF1 or TRAF2 are shown. The TRAF domain was defined in the COOH-terminal region of TRAF1 and TRAF2(19) (residues 356 to 562 for CRAF1) but can be subdivided into TRAF-N and TRAF-C subregions according to sequence homology with CRAF1 as will as by the mapping assaying shown in Fig. 3. For CRAF1, the number of amino acids between homologous regions is indicated. (**B**) Helical wheel representation of residues 287 to 342 of CRAF1. The wheel starts with the inner residue Ile^{287} at position a and diminishes with the outer residue Asn^{342} at position g; "+" and "-" denote change of amino acid residues. (**C**) Predicted Zn fingers corresponding to residues 110 to 264 of CRAF1. (**D**) Zn finger from residues 45 to 106 of CRAF1. n, NH_2-terminus; c, COOH-terminus.

Figure 3. Mapping the CD40 binding and homodimerization domain of CRAF1. C26NX and C26XC represent fragments from the NH_2-terminus of C26 to the internal XhoI site and from the XhoI site to the COOH-terminus of CRAF1, respectively. C26ΔNB was made by deletion of the NcoI-Bgl II fragment in the 3' untranslated region of the C26

cDNA clone. The full TRAF domain of CRAF1 was synthesized by the polymerase chain reaction with the use of plaque-forming units of DNA polymerase. Various DNA fragments were ligated in-frame into yeast expression vectors encoding either the LexA DNA-binding domain (LexA) or the transcriptional activation domain (TAD). For CD40 binding assays, the LexA construct containing the CD40 cytoplasmic tail and various TAD fusion constructs were cotransfected into yeast strain EGY48 along with the lacZ-containing reporter vector (pSH18-34). Colonies that grew up on synthetic dextrose plates without tryptophan, uracil, and histidine were replica-plated to plates with or without leucine and tested for galactose-inducible blue color in the presence of x-gal. LexA constructs containing the cytoplasmic tails of Fas and TNFαRII were also included in the same experiments to test their interaction with the C26 clone. For dimerization assays, various LexA fusion constructs containing different fragments of C26 were used in every combination with various TAD fusion constructs. Transformants that grew on plates lacking leucine and that showed galactose-inducible blue are marked "+"; this was further confirmed by β-galactosidase assays with the use of yeast grown in liquid cultures (34). Transformants that grew only on plates containing leucine but that did not show blue on x-gal plates are marked "-"; ND, experiments not done.

Figures 4A-M. Effect of C26 fusion proteins on CD40L: CD40-induced CD23 up-regulation. (**A**) Northern blot analysis of Ramos 2G6 transfectants. Total RNA (2μg) from the Jurkat T cell line (B2.7) was used for markers. In other lanes, polyadenylate-containing RNA (0.75 μg per lane) was obtained from the untransfected Ramos 2G6 clone (Ramos) or pEBVHis/C26 Ramos transfectants (B6, C5, or D10). RNA blots of control and transfected cell lines were probed with C26 cDNA or an actin probe. (**B-M**)

Two-color fluorescence-activated cell sorting analysis of Ramos 2G6 and Ramos 2G6 transfectants (pEBVHis/C26 or pEBVHis/lacZ) after 18 to 24 hours of culture with medium (-), 293.CD40L cells, rIL-4, or 293.CD40 cells plus anti-CD40L mAb 5C8 (as indicated). The x and y axes represent CD20 and CD23 fluorescence, respectively. The percentage of $CD20^+$ cells that express CD23 is indicated in the upper right-hand corner of each contour map. The D10 clone of pEBVHis/C26 is shown.

Figures 5A-B. cDNA nucleotide sequence and predicted amino acid sequences of mouse CRAF1. The cDNA nucleotide sequence is also deposited in GenBank with accession number U21050.

Figures 6A-B. cDNA nucleotide sequence and predicted amino acid sequences of human CRAF1. The cDNA nucleotide sequence is also deposited in GenBank with accession number U21092.

Detailed Description

This invention provides a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof capable of inhibiting CD40-mediated cell activation. In an embodiment the variant comprises a conservative amino acid substitution.

Variants can differ from naturally occurring CD40 or CD40 ligand in amino acid sequence or in ways that do not involve sequence, or both. Variants in amino acid sequence are produced when one or more amino acids in naturally occurring CD40 or CD40 ligand is substituted with a different natural amino acid, an amino acid derivative or non-native amino acid. When a nucleic acid molecule encoding the protein is expressed in a cell, one naturally occurring amino acid will generally be substituted for another. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics such as substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine; aspartic acid,glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The non-polar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

Other conservative substitutions can be taken from Table 1, and yet others are described by Dayhoff in the Atlas of Protein Sequence and Structure (1988).

Table 1: Conservative Amino Acid Replacements

For Amino Acid	Code	Replace with any of
Alanine	A	D-Ala, Gly,beta-ALa, L-Cys,D-Cys
Arginine	R	D-Arg, Lys,homo-Arg, D-homo-Arg, Met,D-Met, Ile, D-Ile, Orn, D-Orn
Asparagine	N	D-Asn,Asp,D-Asp,Glu,D-Glu, Gln,D-Gln
Aspartic Acid	D	D-Asp,D-Asn,Asn, Glu,D-Glu, Gln, D-Gln
Cysteine	C	D-Cys, S-Me-Cys,Met,D-Met,Thr, D-Thr
Glutamine	Q	D-Gln,Asn, D-Asn,Glu,D-Glu,Asp, D-Asp
Glutamic Acid	E	D-Glu,D-Asp,Asp, Asn, D-Asn, Gln, D-Gln
Glycine	G	Ala, D-Ala,Pro, D-Pro, Beta-Ala, Acp
Isoleucine	I	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	L	D-Leu, Val, D-Val, Met, D-Met
Lysine	K	D-Lys,Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Methionine	M	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val, Norleu
Phenylalanine	F	D-Phe,Tyr, D-Thr,L-Dopa,His,D-His, Trp, D-Trp, Trans 3,4 or 5-phenylproline, cis 3,4 or 5 phenylproline
Proline	P	D-Pro, L-I-thioazolidine-4-carboxylic acid, D- or L-1-oxazolidine-4-carboxylic acid

Serine	S	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Threonine	T	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O) D-Met(O), Val, D-Val
Tyrosine	Y	D-Tyr,Phe, D-Phe, L-Dopa, His,D-His
Valine	V	D-Val, Leu,D-Leu,Ile,D-Ile, Met, D-Met

Other variants within the invention are those with modifications which increase peptide stability. Such variants may contain, for example, one or more non-peptide bonds (which replace the peptide bonds) in the peptide sequence. Also included are: variants that include residues other than naturally occurring L-amino acids, such as D-amino acids or non-naturally occurring or synthetic amino acids such as beta or gamma amino acids and cyclic variants. Incorporation of D- instead of L-amino acids into the polypeptide may increase its resistance to proteases. See, e.g., U.S. Patent No. 5,219,990.

The protein of this invention may also be modified by various changes such as insertions, deletions and substitutions, either conservative or nonconservative where such changes might provide for certain advantages in their use.

In other embodiments, variants with amino acid substitutions which are less conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such substitutions would include for example, substitution of hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for

another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or substitution of a residue having a net positive charge for a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine the presence or absence of the desired characteristics.

Variants within the scope of the invention include proteins and peptides with amino acid sequences having at least eighty percent homology with the COOH-terminal domain of CRAF1 (corresponding roughly to residues 415-567) or with C26 (residues 324-567 of CRAF1). More preferably the sequence homology is at least ninety percent, or at least ninety-five percent.

Just as it is possible to replace substituents of the scaffold, it is also possible to substitute functional groups which decorate the scaffold with groups characterized by similar features. These substitutions will initially be conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group. Non-sequence modifications may include, for example, in vivo or in vitro chemical derivatization of portions of the protein of this invention, as well as changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

In a further embodiment the protein is modified by chemical modifications in which activity is preserved. For example, the proteins may be amidated, sulfated, singly or multiply halogenated, alkylated, carboxylated, or phosphorylated. The protein may also be singly or multiply acylated, such as with an acetyl group, with a farnesyl moiety, or with a fatty acid, which may be

saturated, monounsaturated or polyunsaturated. The fatty acid may also be singly or multiply fluorinated. The invention also includes methionine analogs of the protein, for example the methionine sulfone and methionine sulfoxide analogs. The invention also includes salts of the proteins, such as ammonium salts, including alkyl or aryl ammonium salts, sulfate, hydrogen sulfate, phosphate, hydrogen phosphate, dihydrogen phosphate, thiosulfate, carbonate, bicarbonate, benzoate, sulfonate, thiosulfonate, mesylate, ethyl sulfonate and benzensulfonate salts.

In specific embodiments the CRAF1 is mouse or human CRAF1.

This invention provides a method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, comprising providing the cells with an agent capable of inhibiting CD40-mediated intracellular signaling, the agent being present in an amount effective to inhibit activation of the cells. In an embodiment the agent is a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof.

In an embodiment of the method of inhibiting activation by CD40 ligand of cells bearing CD40 on the cell surface, the cells are provided with the protein of this invention by introducing into the cells a nucleic acid sequence encoding the protein under conditions such that the cells express an amount of the protein effective to inhibit activation of the cells. The nucleic acid may be DNA (including cDNA) or RNA. It may be single or double stranded, linear or circular. It may be in the form of a vector such as a plasmid or a viral vector. Preferably the nucleic acid sequence is operably linked to a transcriptional control sequence recognized by the

host cell.

In another embodiment the agent is a small molecule. As used herein a small molecule is a compound capable of entering the cell. Preferably it has a molecular weight between 20 Da and $1x10^6$ Da, preferably from 50 Da to 2 kDa.

In an embodiment the agent is modified from a lead inhibitory agent. In an embodiment the agent specifically binds to CD40 intracellular domain.

In embodiments of the methods described herein, the CD40-bearing cells are selected from the group consisting of B cells, fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, dendritic cells, renal cells, and smooth muscle cells.

In a more specific embodiment the B cells are resting B cells, primed B cells, myeloma cells, lymphocytic leukemia B cells, or B lymphoma cells. In another specific embodiment the epithelial cells are keratinocytes. In another embodiment the fibroblasts are synovial membrane fibroblasts, dermal fibroblasts, pulmonary fibroblasts, or liver fibroblasts. In another specific embodiment the renal cells are selected from the group consisting of glomerular endothelial cells, mesangial cells, distal tubule cells, proximal tubule cells, parietal epithelial cells (e.g., crescent parietal epithelial cells), visceral epithelial cells, cells of a Henle limb, and interstitial inflammatory cells. In another embodiment the smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells. In a more

specific embodiment the gastrointestinal smooth muscle cells are esophageal smooth muscle cells, stomachic smooth muscle cells, smooth muscle cells of the small intestine, or smooth muscle cells of the large intestine.

This invention provides a method of providing a subject with an amount of the protein of this invention effective to inhibit activation by CD40 ligand of cells bearing CD40 on the cell surface in the subject, comprising: introducing into CD40-bearing cells of the subject, a nucleic acid sequence encoding the protein of this invention, under conditions such that the cells express in the subject an activation inhibiting effective amount of the protein.

In an embodiment of this invention the introducing of the nucleic acid into cells of the subject comprises: a) treating cells of the subject ex vivo to insert the nucleic acid sequence into the cells; and b) introducing the cells from step a) into the subject.

The subject which can be treated by the above-described methods is an animal. Preferably the animal is a mammal. Subjects specifically intended for treatment with the method of the invention include humans, as well as nonhuman primates, sheep, horses, cattle, goats, pigs, dogs, cats, rabbits, guinea pigs, hamsters, gerbils, rats and mice, as well as the organs, tumors, and cells derived or originating from these hosts.

This invention provides a method of treating a condition characterized by an unwanted level of CD40-mediated intracellular signaling, in a subject, comprising providing the subject with an amount of an agent capable of inhibiting CD40-mediated intracellular signaling in cells bearing CD40 on the cell surface.

In an embodiment the agent is a protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof capable of inhibiting CD40-mediated cell activation. In an embodiment the protein is provided by introducing into CD40-bearing cells of the subject, a nucleic acid sequence encoding the protein, under conditions such that the cells express in the subject an activation inhibiting effective amount of the protein.

In an embodiment of this invention the agent is a small molecule. In an embodiment the molecule is modified from a lead inhibitory agent. In an embodiment the agent specifically binds to CD40 intracellular domain.

In an embodiment the condition is organ rejection in a subject receiving transplant organs. Examples of suitable transplant organs include a kidney, heart or liver, as well as others known to those of skill in the art. In another embodiment the condition is an immune response in a subject receiving gene therapy. One difficulty encountered in gene therapy is an immune response by the patient to the gene therapy vector and the proteins it expresses. Because the protein of this invention inhibits the immune response, gene therapy with the protein of this invention does not trigger an immune response. Its immunosuppressant effect also makes it useful as an adjunct to other forms of gene therapy. For example, at the same time that a vector being administered to provide a gene therapy patient with a desired gene product, the patient is also administered a vector which provides the protein of this invention.

In another embodiment the condition is a CD40-dependent immune response. In a specific embodiment the CD40-dependent immune response is an autoimmune response in

a subject suffering from an autoimmune disease, including but not limited to rheumatoid arthritis, Myasthenia gravis, systemic lupus erythematosus, Graves' disease, idiopathic thrombocytopenia purpura, hemolytic anemia, diabetes mellitus, a drug-induced autoimmune disease such as drug-induced lupus, psoriasis, or hyper IgE syndrome.

In another embodiment the condition is an allergic response, including but not limited to hay fever or a penicillin allergy.

In an embodiment of this invention the immune response comprises induction of CD23, CD80 upregulation, or rescue from CD95-mediated apoptosis. Because CD40, which is expressed by many tumors, is involved in rescuing cells from apoptosis, inhibitors of CD40-mediated activity are useful as adjunctive agents in chemotherapy.

In an embodiment of this invention the immune response is autoimmune manifestations of an infectious disease. In more specific embodiments the autoimmune manifestations are derived from Reiter's syndrome, spondyloarthritis, Lyme disease, HIV infections, syphilis or tuberculosis.

In an embodiment the condition is dependent on CD40 ligand-induced activation of fibroblast cells, for example arthritis, scleroderma, and fibrosis. In more specific embodiments the arthritis is rheumatoid arthritis, non-rheumatoid inflammatory arthritis, arthritis associated with Lyme disease, or osteoarthritis. In another specific embodiment the fibrosis is pulmonary fibrosis, hypersensitivity pulmonary fibrosis, or a pneumoconiosis. Examples of pulmonary fibrosis include pulmonary fibrosis secondary

to adult respiratory distress syndrome, drug-induced pulmonary fibrosis, idiopathic pulmonary fibrosis, or hypersensitivity pneumonitis. Examples of pneumoconiosis include asbestosis, siliconosis, or Farmer's lung. In another specific embodiment the fibrosis is a fibrotic disease of the liver or lung, including fibrotic disease of the lung caused by rheumatoid arthritis or scleroderma, and fibrotic diseases of the liver selected from the group consisting of: Hepatitis-C; Hepatitis-B; cirrhosis; cirrhosis of the liver secondary to a toxic insult; cirrhosis of the liver secondary to drugs; cirrhosis of the liver secondary to a viral infection; and cirrhosis of the liver secondary to an autoimmune disease. In a specific embodiment the toxic insult is alcohol consumption. In another specific embodiment the viral infection is Hepatitis B, Hepatitis C, or hepatitis non-B non-C. In another specific embodiment the autoimmune disease is primary biliary cirrhosis, or Lupoid hepatitis.

In an embodiment of this method the condition is dependent on CD40 ligand-induced activation of endothelial cells. In specific embodiments the condition dependent on CD40 ligand-induced activation of endothelial cells is selected from the group consisting of atherosclerosis, reperfusion injury, allograft rejection, organ rejection, and chronic inflammatory autoimmune diseases. In a more specific embodiment the atherosclerosis is accelerated atherosclerosis associated with organ transplantation. In another specific embodiment the chronic inflammatory autoimmune disease is vasculitis, rheumatoid arthritis, scleroderma, or multiple sclerosis.

In an embodiment the condition is dependent on CD40 ligand-induced activation of epithelial cells. In a specific embodiment the epithelial cells are

keratinocytes, and the condition is psoriasis. In another specific embodiment the condition is an inflammatory kidney disease, including inflammatory kidney disease not initiated by autoantibody deposition in kidney and kidney disease which is initiated by autoantibody deposition. In specific embodiments the kidney disease is selected from the group consisting of: membranous glomerulonephritis; minimal change disease/acute tubular necrosis; pauci-immune glomerulonephritis; focal segmental glomerulosclerosis; interstitial nephritis; antitissue antibody-induced glomerular injury; circulating immune-complex disease; a glomerulopathy associated with a multisystem disease; and drug-induced glomerular disease. In an embodiment the antitissue antibody-induced glomerular injury is anti-basement membrane antibody disease. In another embodiment the circulating immune-complex disease is selected from the group consisting of: infective endocarditis; leprosy; syphilis; hepatitis B; malaria; and a disease associated with an endogenous antigen. In a more specific embodiment the endogenous antigen is DNA, thyroglobulin, an autologous immunoglobulin, erythrocyte stroma, a renal tubule antigen, a tumor-specific antigen, or a tumor-associated antigen. In another embodiment the glomerulopathy associated with a multisystem disease is selected from the group consisting of: diabetic nephropathy; systemic lupus erythematosus; Goodpasture's disease; Henoch-Schönlein purpura; polyarteritis; Wegener's granulomatosis; cryoimmunoglobulinemia; multiple myeloma; Waldenström's macroglobulinemia; and amyloidosis. In an embodiment the pauci-immune glomerulonephritis is ANCA+ pauci-immune glomerulonephritis, or Wegener's granulomatosis. In an embodiment the interstitial nephritis is drug-induced interstitial nephritis. In another embodiment the kidney disease affects renal tubules, including but not limited to: a kidney disease associated with a

toxin; a neoplasia; hypersensitivity nephropathy; Sjögren's syndrome; and AIDS.

In an embodiment the condition is a smooth muscle cell-dependent disease. Examples include vascular diseases such as atherosclerosis; gastrointestinal diseases such as esophageal dysmotility, inflammatory bowel disease, and scleroderma; and bladder diseases.

In an embodiment of this method, the condition is associated with Epstein-Barr virus. Examples of Epstein-Barr virus-associated conditions include mononucleosis, B cell tumors (particularly in immunosuppressed individuals such as chemotherapy patients and those with acquired immune deficiency syndrome (AIDS)), Burkitt's lymphoma, and nasopharyngeal carcinoma. Epstein-Barr virus (EBV) transforms cells using latent infection membrane protein 1 (LMP1). LMP1 binds to CRAF1 (also known as LAP1)(33).

This invention provides a nucleic acid molecule encoding the protein of this invention. The nucleic acid may be DNA (including cDNA) or RNA. It may be single or double stranded, linear or circular. It may be in the form of a vector, such as a plasmid or viral vector, which comprises the nucleic acid molecule operably linked to a transcriptional control sequence recognized by a host cell transformed with the vector.

This invention provides a method of identifying an agent capable of inhibiting CD40-mediated intracellular signaling in a cell expressing CD40 on the cell surface, comprising providing the cell with the agent under conditions permitting activation of the cell in the absence of the agent, and determining decreased or absent activation, thereby identifying an agent capable of inhibiting CD40-mediated intracellular signaling in

a cell expressing CD40 on the cell surface. In an embodiment the activation comprises up-regulation of CD23. In an embodiment the conditions permitting activation of the cell comprises contacting the cell with CD40 ligand or portion thereof effective to activate the cell.

This invention will be better understood from the Experimental Details which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention as described more fully in the claims which follow thereafter.

Experimental Details

Activity of N-terminal Truncated CRAF1

The yeast two-hybrid system was used to identify complementary DNAs (cDNAs) encoding protein domains that can bind to the tail. The bait for the yeast two-hybrid screen was a LexA fusion protein containing the cytoplasmic tail of the mouse CD40 receptor (from residue 219 to the COOH-terminus).

The cDNA library for the yeast two-hybrid screen was a mixture of oligo(dT) and random primed cDNAs constructed into the yeast expression vector YSD, which is a centromere-based, galactose-induced yeast expression vector containing the VP16 transcription activation domain. Half of the mRNA used for cDNA synthesis was isolated from uninduced 70Z cells, and the other half was from 70Z cells that were induced with lipopolysaccharide for 12 hours. The primary library contained about 8 x 10^5 individual clones, with an average insert size of 0.9 kb. From 2 x 10^6 clones of the murine 70Z pre-B cell cDNA library, one (C26) was

isolated that met all specificity criteria for binding to the cytoplasmic tail of CD40 in yeast. The C26 cDNA fragment was sequenced and no identical gene was evident in the databases. This gene is called CRAF1 for CD40 receptor-associated factor 1. By Northern (RNA) blot analysis, CRAF1 was expressed in B cell lines representing different stages of B cell differentiation; in addition, it was expressed in all murine tissues examined, including brain, heart, lung, liver, kidney, muscle, small intestine, spleen, and thymus (18).

Mouse and human cDNA libraries were screened to isolate cDNA clones encoding the entire open reading frame of a murine 567-amino acid and a human 568-amino acid protein. The two sequences share 96% identity, with the differences concentrated near the NH_2-terminus, indicating that CRAF1 is evolutionarily conserved, particularly in its COOH-terminal 400 amino acids (Fig. 1). The CRAF1 sequence is similar to that of TNF-α receptor-associated factors 1 and 2 (TRAF1 and TRAF2), which can complex with the cytoplasmic tail of TNF-α receptor II (TNFαRII) (19). The COOH-terminus of CRAF1 is related by sequence to each of these TRAF proteins for 150 amino acids, wherein CRAF1 is 59 and 62% identical to TRAF1 or TRAF2, respectively (Fig. 2) (19). This homology subdivides what was termed the "TRAF domain," excluding a more NH_2-terminal putative coiled-coiled subdomain (TRAF-N) with which CRAF1 shares only 16 or 12% homology and defines a "TRAF-C" (for COOH-terminal) domain. Because the extracellular domains of CD40 and TNFαRII are homologous, as are their ligands, these data suggest that they may make use of related but distinct signaling molecules. However, the cytoplasmic domains of CD40 and TNFαRII contain no apparent sequence homology, which suggests that the particular contacts involved in binding the signaling molecules to the receptors have diverged.

In addition to the TRAF-C domain, sequence analysis of the CRAF1 protein revealed three potential domains: an amphipathic helix, a string of Zn fingers, and a Zn ring finger domain (Fig. 2A). A helical wheel representation of the putative helix (Fig. 2B) shows that isoleucine (or occasionally leucine) repeats every seven residues through eight consecutive repeats, which implies the presence of an isoleucine zipper in analogy to the leucine zipper seen in other proteins (20). The wheel also indicates that the position next to the zipper is always hydrophobic or uncharged, whereas the other positions around the wheel include multiple charged residues and few hydrophobic ones. This strongly suggests an amphipathic structure that could be an interaction site for another such helix.

The are five repeats of potential Zn fingers just NH_2-terminal to the isoleucine repeats (Fig. 2C). However, the four amino acids that would contact the metal are arranged in the unique pattern Cys-X_{2-6}-Cys-$X_{11,12}$-His-X_{3-7}-Cys(His), instead of Cys-X_{2-4}-Cys-$X_{12,13}$-His-X_{2-4}-His, which is seen in classic Zn fingers (21). At the COOH-terminal edge of finger 2 is a sequence (KACKYR) that could bind to DNA, which suggests that CRAF1 might be a DNA binding protein. The TRAF2 protein contains five fingers with the same pattern of repeats seen in the CRAF1 protein but with weak overall similarity (Fig.2A), suggesting that these structural units may represent a subclass of Zn finger motifs in this type of signaling molecule. In addition, a Zn ring structure was also evident in the NH_2-terminus of CRAF1 (Fig. 2D) (23). This ring motif has been recognized in over 40 proteins that have diverse functions related to DNA mechanics, including recombination, repair, and transcription regulation (24). These structural data suggest that CRAF1 directly transmits CD40 signals to the nucleus.

To further map the region of CRAF1 that interacts with the CD40 cytoplasmic tail, four deletion mutants of the C26 cDNA were generated and studied in the yeast system for their ability to bind to the CD40 cytoplasmic tail. The TRAF-C subdomain of CRAF1 was necessary and sufficient for CRAF1 to interact with CD40 (Fig. 3). Moreover, the CRAF1 protein in yeast could interact with itself, forming homodimers or oligomers, also mediated by the TRAF-C domain (Fig. 3). Quantitative analysis of β-galactosidase expression indicated that the affinity of the TRAF-C domain of CRAF1 to bind to CD40 and to dimerize with itself was not increased by addition of the rest of the TRAF domain. These data suggest that the COOH-terminal portion of the TRAF domain functions as an individual unit (the TRAF-C domain) that is involved in both binding to the receptor tail and mediating dimerization.

Overexpression of the C26 partial cDNA fragment acts as a dominant negative protein, inhibiting CD40 signaling presumably by prevention of the binding of the endogenous protein to the CD40 tail. Ramos 2G6 cells (25) can be induced to up-regulate surface CD23 molecules in a contact-dependent fashion that depends on CD40L interaction with CD40 (3). Therefore, a cDNA construct was generated that drives the expression of a polyhistidine/C26 fusion protein (pEBVHis/C26) in mammalian cells. The C26 cDNA fragment was cut with Eco RI-Hinc III from yeast vector YSD, ligated into Bluescript IISK+ (Stratagene), and then recloned in-frame into the pEBVHisA vector (Invitrogen), with the use of Bam Hl and Kpn 1 cuts, to create pEBVHis/C26. Stable Ramos cell lines containing either this construct or the control construct (pEBVHis/lacZ) were isolated by electroporation and hygromycin selection.

As a negative control for the effects of C26, the β-

galactosidase gene was expressed as a fusion protein in the same vector (pEB-VHis/lacZ) (Invitrogen). These constructs were electroporated into Ramos 2G6 cells, and clones expressing a large amount of pE-BVHis/C26 mRNA were prepared (Fig. 4A). CD40L-expressing cells (293.CD40L) were then cultured with Ramos 2G6 cells that either were not transfected or were stably expressing pEBVHis/lacZ or pEBVHis/C26. Either 2 x 10^5 Ramos B cells or Ramos B cells transfected with pEBVHis/C26 or pEBVHis/lacZ were incubated for 18 to 24 hours in 0.2 ml of medium alone, in rIL-4 at a concentration of 25 ng/ml, or in the presence of 5 x 10^4 293.CD40L cells. In some cases, mAb 5C8 (anti-CD40L) was added. Cells were then washed and incubated with saturating concentrations of mAb Leu-16 (anti-CD20) conjugated to fluorescein isothiocyanate (Becton Dickinson) and mAb to CD23 conjugated to phycoerythrin (Biosource International) for 45 min at 4°C in the presence of heat-aggregated IgG (80 μg/ml) (International Enzyme). Cells were washed to remove unbound antibody before fluorescence intensity was measured on a FACSCAN cytofluorograph (Becton Dickinson) with Consort 30 software.

The control and pEBVHis/lacZ-transfected Ramos lines up-regulated CD23; this effect was inhibited by a monoclonal antibody (mAb) to CD40L (mAb 5C8). In contrast, the ability of the pEBVHis/C26 transfectants to up-regulate CD23 in response to CD40L-CD40 signals was diminished. The inhibition of CD23 up-regulation by pEB-VHis/C26 was relatively specific because recombinant interleukin-4 (rIL-4)-induced up-regulation of CD23 was not affected (Fig. 4B-M). Similar effects were seen in all three subclones of pEBVHis/C26 transfectants. Thus, the COOH-terminal region of CRAF1 represented in the C26 cDNA could block the CD40 triggering of Ramos cells.

CD40 is a type I transmembrane glycoprotein belonging to

the TNF receptor superfamily. Besides CD40, 11 other proteins have been identified in this superfamily, which includes TNF receptors I and II, the nerve growth factor (NGF) receptor, and Fas (28). Members within this family share sequence similarity through their extracellular regions that contain multiple cysteine-rich pseudorepeats. The common structural framework of the extracellular domain is reflected in the ability of the TNF receptor superfamily members to interact with a parallel family of TNF-related cytokine ligands. Eight such ligands (including TNF-α, CD40L, and FasL) have been cloned that share extensive sequence identity and exist as secreted cytokines or type II transmembrane ligands (28).

The functions of TNF receptor superfamily members are very divergent. They range from general acute phase response and lymphocyte activation to nerve cell growth. In some circumstances, they have opposite roles. For instance, Fas and TNFαRI can cause apoptosis upon ligand stimulation, whereas CD40 and NGF receptors can rescue cells from apoptosis (29). In addition, stimulation of either TNFαRI, TNFαRII, or CD40 receptor activates nuclear factor kappa B (30). Because CRAF1 is very similar to TRAF1 and TRAF2, a family of signal transduction proteins (the TRAF family) probably exists as downstream signal transducers of the TNF receptor superfamily. It is likely that direct binding between members of the TNF receptor family and the TRAF family will be specific because the cytoplasmic tails of these TNF receptor superfamily members are relatively short and show little or no sequence homology. Consistent with this notion, the COOH-terminal segment of CRAF1 does not interact with the tail of Fas or with TNFαRII (Fig. 3). However, the fact that the members of the TRAF family can form either homodimers or heterodimers could result in extensive diversity and specificity in

their signal transduction pathways. It is even possible that apoptosis and cell survive may be determined by an equilibrium of dimerization between TRAF family members.

The functional consequences of CD40 signaling are different for B cells at different stages of differentiation (31). CD40 crosslinking causes resting B cells to enter into the cells cycle, enhancing the proliferative rate of some chronic lymphocytic leukemia B cells, induces some B lymphoma cells to apoptose, and prevents germinal center B cells from apoptosis (14). However, CRAF1 is expressed at all stages of B cell differentiation and may be ubiquitous.

Gene Therapy

The invention features expression vectors for in vivo transfection and expression in particular cell types of CD40 receptor-associated factor truncated at the amino terminus so as to antagonize the function of wild type CD40 receptor-associated factor in an environment in which the wild-type protein is expressed (i.e., introduce abnormal CD40 receptor-associated factor that acts as a dominant negative protein to inhibit CD40 signaling).

Expression constructs of CD40 receptor-associated factor polypeptides may be administered in any biologically effective carrier that is capable of effectively delivering a polynucleotide sequence encoding the CD40 receptor-associated factor to cells in vivo. Approaches include insertion of the subject gene in viral vectors including recombinant retroviruses, adenovirus, adeno-associated virus and herpes simplex virus-1, or recombinant bacterial or eukaryotic plasmids. Viral vectors transfect cells directly, plasmid DNA can be delivered with the help of, for , example, cationic

liposomes or derivatized (e.g., antibody conjugated) polylysine conjugates, gramacidin S, artificial viral envelopes or other such intracellular carriers, as well as direct injection of the gene construct or $CaPO_4$ precipitation carried out in vivo.

Any of the methods known in the art for the insertion of polynucleotide sequences into a vector may be used. See, for example, Sambrook et al., **Molecular Cloning: A Laboratory Manual**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989) and Ausubel et al., **Current Protocols in Molecular Biology**, J. Wiley & Sons, NY (1992), both of which are incorporated herein by reference. Conventional vectors consist of appropriate transcriptional/translational control signals operatively linked to the polynucleotide sequence for a particular anti-fibrotic polynucleotide sequence . Promoters/enhancers may also be used to control expression of anti-fibrotic polypeptide. Promoter activation may be tissue specific or inducible by a metabolic product or administered substance. Such promoters/enhancers include, but are not limited to, the native E2F promoter, the cytomegalovirus immediate-early promoter/enhancer (Karasuyama et al., *J. Exp. Med.*, 169: 13 (1989)); the human beta-actin promoter (Gunning et al., *Proc. Natl. Acad. Sci. USA*, 84: 4831 (1987); the glucocorticoid-inducible promoter present in the mouse mammary tumor virus long terminal repeat (MMTV LTR) (Klessig et al., *Mol. Cell. Biol.*, 4: 1354 (1984)); the long terminal repeat sequences of Moloney murine leukemia virus (MuLV LTR) (Weiss et al., **RNA Tumor Viruses**, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1985)); the SV40 early region promoter (Bernoist and Chambon, *Nature*, 290:304 (1981)); the promoter of the Rous sarcoma virus (RSV) (Yamamoto et al., *Cell*, 22:787 (1980)); the herpes simplex virus (HSV) thymidine kinase promoter (Wagner et al., *Proc.*

Natl. Acad. Sci. USA, 78: 1441 (1981)); the adenovirus promoter (Yamada et al., *Proc. Natl. Acad. Sci. USA*, 82: 3567 (1985)).

Expression vectors compatible with mammalian host cells for use in gene therapy of tumor cells include, for example, plasmids; avian, murine and human retroviral vectors; adenovirus vectors; herpes viral vectors; and non-replicative pox viruses. In particular, replication-defective recombinant viruses can be generated in packaging cell lines that produce only replication-defective viruses. See **Current Protocols in Molecular Biology**: Sections 9.10-9.14 (Ausubel et al., eds.), Greene Publishing Associcates, 1989.

Specific viral vectors for use in gene transfer systems are now well established. See for example: Madzak et al., *J. Gen . Virol.*, 73: 1533-36 (1992: papovavirus SV40); Berkner et al., *Curr. Top. Microbiol. Immunol.*, 158: 39-61 (1992: adenovirus); Moss et al., *Curr. Top. Microbiol. Immunol.*, 158: 25-38 (1992: vaccinia virus); Muzyczka, *Curr. Top. Microbiol. Immunol.*, 158: 97-123 (1992: adeno-associated virus); Margulskee, *Curr. Top. Microbiol. Immunol.*, 158: 67-93 (1992: herpes simplex virus (HSV) and Epstein-Barr virus (HBV)); Miller, *Curr. Top. Microbiol. Immunol.*, 158: 1-24 (1992:retrovirus); Brandyopadhyay et al., *Mol. Cell. Biol.*, 4: 749-754 (1984: retrovirus); Miller et al., *Nature*, 357: 455-450 (1992: retrovirus); Anderson, *Science*, 256: 808-813 (1992:retrovirus), all of which are incorporated herein by reference.

Preferred vectors are DNA viruses that include adenoviruses (preferably Ad-2 or Ad-5 based vectors), herpes viruses (preferably herpes simplex virus based vectors), and parvoviruses (preferably "defective" or non-autonomous parvovirus based vectors, more preferably

adeno-associated virus based vectors, most preferably AAV-2 based vectors). See, e.g., Ali et al., *Gene Therapy* 1: 367-384, 1994; U.S. Patent 4,797,368 and 5,399,346 and discussion below.

Furthermore, abnormal or wild-type CD40 receptor-associated factor may also be introduced into a target cell using a variety of well-known methods that use non-viral based strategies that include electroporation, membrane fusion with liposomes, high velocity bombardment with DNA-coated microprojectiles, incubation with calcium-phosphate-DNA precipitate, DEAE-dextran mediated transfection, and direct micro-injection into single cells. For instance, an anti-fibrotic polynucleotide encoding an immunosuppressant effective amount of an abnormal CD40 receptor-associated factor may be introduced into a cell by calcium phosphate coprecipitation (Pillicer et al., *Science*, 209: 1414-1422 (1980); mechanical microinjection and/or particle acceleration (Anderson et al., *Proc. Natl. Acad. Sci. USA*, 77: 5399-5403 (1980); liposome based DNA transfer (e.g., LIPOFECTIN-mediated transfection- Fefgner et al., *Proc. Natl. Acad. Sci. USA*, 84: 471-477 (1987), Gao and Huang, *Biochem. Biophys. Res. Comm.*, 179: 280-285, 1991); DEAE Dextran-mediated transfection; electroporation (U.S. Patent 4,956,288); or polylysine-based methods in which DNA is conjugated to deliver DNA preferentially to liver hepatocytes (Wolff et al., *Science*, 247: 465-468 (1990), Curiel et al., *Human Gene Therapy* 3: 147-154 (1992). Each of these methods is well represented in the art. Moreover, plasmids containing isolated polynucleotide sequences encoding CD40 receptor-associated factor polypeptide may placed into cells using many of these same methods.

CD40 receptor-associated factor itself may also be chemically modified to facilitate its delivery to a

target cell. One such modification involves increasing the lipophilicity of the CD40 receptor-associated factor in order to increase cell surface binding and stimulate non-specific endocytosis of the polypeptide. A wide variety of lipopeptides, fatty acids, and basic polymers (e.g., tripalmitoyl-S-glycerylcysteil-seryl-serine; palmitic acid; polyarginine) may be linked to an anti-fibrotic polypeptide to accomplish this. See U.S. Patent 5, 219,990, incorporated herein by reference.

Delivery may also be effected by using carrier moieties known to cross cell membranes. For example, an abnormal CD40 receptor-associated factor may be fused to a carrier moiety, preferably by genetic fusion, and the fused construct may be expressed in bacteria or yeast using standard techniques. Thus, polynucleotide sequences encoding abnormal or wild type CD40 receptor-associated factor useful in the present invention, operatively linked to regulatory sequences, may be constructed and introduced into appropriate expression systems using conventional recombinant DNA techniques. The resulting fusion protein may then be purified and tested for its capacity to enter intact target cells and inhibit growth of the target cells once inside the target. For example, recombinant methods may be used to attach a carrier moiety to anti-fibrotic polynucleotide sequences by joining the polynucleotide sequence encoding for abnormal CD40 receptor-associated factor with the polynucleotide sequence encoding a carrier moiety and introducing the resulting construct into a cell capable of expressing the conjugate. Two separate sequences may be synthesized, either by recombinant means or chemically, and subsequently joined using known methods. The entire conjugate may be chemically synthesized as a single amino acid sequence.

Useful carrier moieties include, for example, bacterial

hemolysins or "blending agents" such as alamethicin or sulfhydryl activated lysins. Other carrier moieties include cell entry components of bacterial toxins such as Pseudomonas exotoxin, tetanus toxin, ricin toxin and diphtheria toxin. Other useful carrier moieties include proteins which are viral receptors, cell receptors or cell ligands for specific receptors that are internalized and cross mammalian cell membranes via specific interaction with cell surface receptors. Such cell ligands include epidermal growth factor, fibroblast growth factor, transferrin and platelet derived growth factor. The carrier moiety may also include bacterial immunogens, parasitic immunogens, viral immunogens, immunoglobulins, and cytokines.

In one embodiment, purified human immunodeficiency virus type-1 (HIV) tat protein is the carrier moiety. Purified human immunodeficiency virus type-1 (HIV) tat protein is taken up from the surrounding medium by human cells growing in culture. See Frankel et al., *Cell* 55: 1189-1193, (1988); Fawell et al., *Proc. Natl. Acad. Sci. USA*, 91: 664-668 (1994)(use of tat conjugate); and Pepinsky et al., *DNA and Cell Biology*, 13: 1011-1019 (1994)(use of tat genetic fusion construct), all of which are incorporated herein by reference. See also PCT Application Serial Number PCT/US93/07833, published 3 March 1994 which describes the tat-mediated uptake of the papillomavirus E2 repressor; utilizing a fusion gene in which the HIV-1 tat gene is linked to the carboxy-terminal region of the E2 repressor open reading frame. The tat protein can deliver, for example, abnormal or wild type CD40 receptor-associated factor and polynucleotide sequences into cells, either in vitro or in vivo. For example, delivery can be carried out in vitro by adding a genetic fusion encoding an abnormal CD40 receptor-associated factor- tat conjugate to cultured cells to produce cells that synthesize the tat

conjugate or by combining a sample (e.g., blood, bone marrow, tumor cell) from an individual directly with the conjugate, under appropriate conditions. The target cells may be in vitro cells such as cultured animal cells, human cells or microorganisms. Delivery may be carried out in vivo by administering the CD40 receptor-associated factor and tat protein to an individual in which it is to be used. The target may be in vivo cells, i.e., cells composing the organs or tissue of living animals or humans, or microorganisms found in living animals or humans. The ADP ribosylation domain from *Pseudomonas* exotoxin ("PE") and pancreatic ribonuclease have been conjugated to tat to confirm cytoplasmic delivery of a protein. The ADP phosphorylation domain is incapable of entering cells so that cytoplasmic delivery of this molecule would be confirmed if cell death occurs. Likewise, ribonuclease itself is incapable of entering cells so that inhibition of protein synthesis would be a hallmark of intracellular delivery using a tat conjugate.

Chemical (i.e., non-recombinant) attachment of CD40 receptor-associated factor polypeptide sequences to a carrier moiety may be effected by any means which produces a link between the two components which can withstand the conditions used and which does not alter the function of either component. Many chemical cross-linking agents are known and may be used to join an abnormal or wild-type CD40 receptor-associated factor polynucleotide sequence or polypeptide to carrier moieties. Among the many intermolecular cross-linking agents are, for example, succinimidyl 3-(2-pyridyldithio)propionate (SPDP) or N, N'-(1,2-phenylene)bismaleimide are highly specific for sulfhydryl groups and form irreversible linkages; N, N'-ethylene-bis-(iodoacetamide) (specific for sulfhydryl); and 1,5-difluoro-2,4-dinitrobenzene (forming

irreversible linkages with tyrosine and amino groups). Other agents include p,p'-difluoro-m,m'-dinitrodiphenylsulfone (forming irreversible linkages with amino and phenolic groups); dimethyl adipimidate (specific for amino groups); hexamethylenediisocyante (specific for amino groups); disdiazobenzidine (specific for tyrosine and histidine); succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC); m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS); and succinimide 4-(p-maleimidophenyl)butyrate (SMPB). The succinimidyl group of these cross-linkers reacts with a primary amine, and the thiol-reactive maleimide reacts with the thiol of a cysteine residue. See, Means and Feeney, **Chemical Modification of Proteins**, Holden-Day, 39-43, 1974; and S.S. Wong, **Chemistry of Protein Conjugation and Cross-Linking**, CRC Press, 1971. All the cross-linking agents discussed herein are commercially available and detailed instructions for their use are available from the suppliers.

In clinical settings, the delivery systems for the abnormal or wild-type CD40 receptor-associated factor polynucleotide sequence can be introduced into a patient by any number of methods, each of which is familiar to persons of ordinary skill. Specific incorporation of the delivery system in the target cells occurs primarily from specificity of transfection provided by the gene delivery vehicle, cell type or tissue type expression due to the transcriptional regulatory sequences controlling expression of the polynucleotide, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being localized by, for example, catheter (U.S. Patent 5,328,470) or stereotactic injection (Chen et al., *Proc. Natl. Acad. Sci. USA*, 91: 3054-3057 (1994).

The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is embedded. Where the complete gene delivery system can be produced intact from recombinant cells such as retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Effective amounts of the compounds of the invention may be administered in any manner which is medically acceptable. The method of administration may include injections, by parenteral routes such as intravenous, intravascular, intraarterial, subcutaneous, intramuscular, intratumor, intraperitoneal, intraventricular, intraepidural, or others as well as oral, nasal, ophthalmic, rectal, topical, or inhaled. The term "pharmaceutically acceptable carrier" means one or more organic or inorganic ingredients, natural or synthetic, with which the molecule is combined to facilitate its application. A suitable carrier includes sterile saline although other aqueous and non-aqueous isotonic sterile solutions and sterile suspensions known to be pharmaceutically acceptable are known to those of ordinary skill in the art. In this regard, the term "carrier" encompasses liposomes or the HIV-1 tat protein (See Pepinsky et al., supra) as well as any plasmid and viral expression vectors. An "effective amount" refers to that amount which is capable of ameliorating or delaying progression of the diseased, degenerative or damaged condition. An effective amount can be determined on an individual basis and will be based, in part, on consideration of the symptoms to be treated and results sought. An effective amount can be determined by one of ordinary skill in the art employing such factors and using no more than routine experimentation.

In preferred methods, an effective amount of the abnormal or wild-type CD40 receptor-associated factor or polynucleotide sequence encoding the factor (contained within its attendant vector; i.e., "carrier) may be directly administered to a target cell or tissue via direct injection with a needle or via a catheter of other delivery tube placed into the cell or tissue. Dosages will depend primarily on factors such as the condition being treated, the selected polynucleotide, the age, weight, and health of the subject, and may thus vary among subjects. An effective amount for a human subject is believed to be in the range of about 0.1 to about 50 ml of saline solution containing from about 1 x 10^7 to about 1 x 10^{11} plaque forming units (pfu)/ml CD40 receptor-associated factor polynucleotide containing, viral expression vectors.

Target cells treated by abnormal or wild-type CD40 receptor-associated factor polynucleotide sequences may be administered topically, intraocularly, parenterally, intranasally, intratracheal, intrabronchially, intramuscularly, subcutaneously or by any other means. Target cells to be treated by abnormal or wild-type CD40 receptor-associated factor protein may be administered topically, intraocularly, parenterally, intranasally, intratracheal, intrabronchially, intramuscularly, subcutaneously or by any other means.

The protein compounds of the invention are administered at any dose per body weight and any dosage frequency which is medically acceptable. Acceptable dosage includes a range of between about 0.01 and 500 mg/kg subject body weight. A preferred dosage range is between about 1 and 100 mg/kg. Particularly preferred is a dose of between about 1 and 30 mg/kg. The dosage is repeated at intervals ranging from each day to every other month. One preferred dosing regime is to

administer a compound of the invention daily for the first three days of treatment, after which the compound is administered every 3 weeks, with each administration being intravenously at 5 or 10 mg/kg body weight. Another preferred regime is to administer a compound of the invention daily intravenously at 5 mg/kg body weight for the first three days of treatment, after which the compound is administered subcutaneously or intramuscularly every week at 10 mg per subject.

The protein compounds of the invention, similarly to the therapeutic nucleotide sequences, may be delivered to tissues in a liposome-encapsulated formulation, or conjugated to carrier moieties such as IIIV tat protein. This delivery can be systemic, such as by intravascular delivery, or local. Local means of delivery of liposome-encapsulated compounds of the invention include intratumor or intraorgan injection. It also includes local delivery by catheter, such as intrahepatic delivery into the portal vein, intrarenal or intraprostate delivery via the urethra, intracholecystic delivery via the bile duct, or delivery into various blood vessels of interest, particularly the coronary vessels or sites of vascular stenosis. Targeted delivery may be accomplished by inserting components into the surface of the liposomes or other carrier moieties which confer target specificity. For example, areas of inflammation might be targeted by coating the carrier liposomes with monoclonal antibodies specific for anti-CD40 ligand. Various types of tumors could be selectively targeted by coating liposomes with monoclonal antibodies specific for surface antigens characteristic of the tumor cells.

The compounds of the invention may be administered as a single dosage for certain indications such as preventing immune response to an antigen to which a subject is

exposed for a brief time, such as an exogenous antigen administered on a single day of treatment. Examples of such an antigen would include coadministration of a compound of the invention along with a gene therapy vector, or a therapeutic agent such as an antigenic pharmaceutical or a blood product. In indications where antigen is chronically present, such as in controlling immune reaction to transplanted tissue or to chronically administered antigenic pharmaceuticals, the compounds of the invention are administered at intervals for as long a time as medically indicated, ranging from days or weeks to the life of the subject.

REFERENCES

1. S. Paulie et al., Cancer Immunol. Immunother. **20**, 23(1985); E.A. Clark and J.A. Ledbetter, Proc. Natl. Acad. Sci. U.S.A. **83**, 4494 (1986); I. Stamenkovic, E.A. Clark, B. Seed, EMBO J. **8**, 1403 (1989).
2. S. Lederman et al., J. Exp. Med. **175**, 1091 (1992); R. J. Armitage et al., Nature **357**, 80(1992); D. Graf, U. Korthauer, H.W. Mages, G. Senger, R.A. Kroczek, Eur. J. Immunol. **22**, 3191 (1992); J.F. Gauchat et al., FEBS Lett. **315**, 259 (1993).
3. S. Lederman et al., J. Immunol. **149**, 3817 (1992).
4. D. Hollenbaugh et al., EMBO J. **11**, 4313 (1992).
5. T. Tsukata, J. Wu, T. Honjo, Nature **364**, 645 (1993); N.J. Holder et al., Eur. J. Immunol. **23**, 2368 (1993); S.L. Parry, J. Hasbold, M. Holman, G.G.B. Klaus J. Immunol. **152**, 2821 (1994).
6. M.J. Yellin et al., J. Immunol. **153**, 666 (1994).
7. R.C. Allen et al., Science **259**, 990 (1993); U. Korthäuer et al., Nature **361**, 539 (1993); J.P. DiSanto et al., ibid., p.541; A. Aruffo et al., Cell **72**, 291 (1993); R.E. Callard et al., J. Immunol. **153**, 3295 (1994); N. Ramesh et al., Int. Immunol. **5**, 769 (1993).
8. J. Xu et al., Immunity **1**, 423 (1994).
9. T. Kawabe et al., ibid., p. 167.
10. F. Schrieve et al., J. Exp. Med. **169**, 2043 (1989); E.A. Clark, K.H. Grabstein, G.L. Shu, J. Immunol. **148**, 3327 (1992).
11. D.N. Hart and J.L. McKenzie, J. Exp. Med. **168**, 157 (1988).
12. M.R. Alderson et al., ibid. **178**, 669 (1993).
13. A.H. Galy and H. Spits, J. Immunol. **149**, 775 (1992).
14. F.H. Durie, T.M. Foy, S.R. Masters, J.D. Laman, R.J. Noelle, Immunol. Today **15**, 406 (1994).

15. S. Inui et al., Eur. J. Immunol. **20**, 1747 (1990).

19. M. Rothe, S.C. Wong, W.J. Henzel, D.V. Goeddel, Cell **78**, 681 (1994).

20. W.H. Landschulz, P.F. Johnson, S.L. McKnight, Science **240**, 1759 (1988).

21. R.M. Evans and S.M. Hollenberg, Cell **52**, 1 (1988).

23. P.S. Fremont, I.M. Hanson, J. Trowsdale, ibid. **64**, 483 (1991).

24. P.N. Barlow, B. Luisi, A. Milner, M. Elliot, R. Everett, J. Mol. Biol. **237**, 201 (1994).

25. J.P. Siegel and H.S. Mostowski, J. Immunol. Methods **132**, 287 (1990).

28. C.A. Smith, T. Farrah, R.G. Goodwin, Cell **76**, 959 (1994).

29. N. Itoh et al., ibid. **66**, 233 (1991); L.A. Taraglis, T.M. Aryes. G.H. W. Wong, D.V. Goeddel. Ibid. **74**, 845 (1993).

30. M. Grilli, J.J-S. Chiu, M.J. Lenardo, Int. Rev. Cytol. **143**, 1(1991); I. Berberich, G.L. Shu, E.A. Clark, J. Immunol. **153**, 4357 (1994).

31. J. Gordon, M.J. Millsum, G.R. Guy, J.A. Ledbetter, J. Immunol. **140**, 1425 (1988); M.F. Gruber, J.M. Bjorndahl, S. Nakamuar, S.M. Fu, ibid. **142**, 4144 (1989); M.K. Spriggs et al., J. Exp. Med. **176**, 1543 (1992); P. Lane et al., ibid. **177**, 1209 (1993); D.H. Crawford and D. Catovsky, Immunology **80**, 40 (1993); A.W. Heath et al., Cell Immunol. **152**, 468 (1993); Y.J. Liu et al., Nature **342**, 929 (1989).

32. H.M. Hu, K. O'Rourke, M.S. Boguski, V.M. Dixit, J. Biol. Chem. **269**, 30069 (1994).

33. G. Mosialos et al., Cell **80**, 389 (1995).

34. J.H. Miller, in Experiments in Molecular Genetics, C. S.H.L. Press, Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1972).

What is claimed is:

1. A protein comprising CRAF1 truncated by from about 323 to about 414 amino acid residues at the amino terminus, or a variant thereof capable of inhibiting CD40-mediated cell activation.

2. The protein of claim 1, wherein the variant comprises a conservative amino acid substitution.

3. The protein of claim 1, wherein the CRAF1 is mouse CRAF1.

4. The protein of claim 1, wherein the CRAF1 is human CRAF1.

5. A method of inhibiting activation by CD40 ligand of cells expressing CD40 on the cell surface, comprising providing the cells with an agent capable of inhibiting CD40-mediated intracellular signaling, the agent being present in an amount effective to inhibit activation of the cells.

6. The method of claim 5, wherein the agent is the protein of claim 1.

7. The method of claim 6, wherein the cells are provided with the protein by introducing into the cells a nucleic acid sequence encoding the protein under conditions such that the cells express an amount of the protein effective to inhibit activation of the cells.

8. The method of claim 7, wherein the nucleic acid sequence is operably linked to a transcriptional control sequence recognized by the cell.

9. The method of claim 7, wherein the nucleic acid sequence is a plasmid.

10. The method of claim 5, wherein the agent is a small molecule.

11. The method of claim 5, wherein the agent is modified from a lead inhibitory agent.

12. The method of claim 5, wherein the agent specifically binds to CD40 intracellular domain.

13. The method of claim 5, wherein the CD40-bearing cells are selected from the group consisting of B cells, fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, dendritic cells, renal cells, and smooth muscle cells.

14. The method of claim 13, wherein the B cells are resting B cells, primed B cells, myeloma cells, lymphocytic leukemia B cells, or B lymphoma cells.

15. The method of claim 5, wherein the epithelial cells are keratinocytes.

16. The method of claim 5, wherein the fibroblasts are synovial membrane fibroblasts, dermal fibroblasts, pulmonary fibroblasts, or liver fibroblasts.

17. The method of claim 5, wherein the renal cells are selected from the group consisting of glomerular endothelial cells, mesangial cells, distal tubule cells, proximal tubule cells, parietal epithelial cells, visceral epithelial cells, cells of a Henle limb, and interstitial inflammatory cells.

18. The method of claim 16, wherein the parietal epithelial cells are crescent parietal epithelial cells.

19. The method of claim 5, wherein the smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, aortic smooth muscle cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells.

20. The method of claim 19, wherein the gastrointestinal smooth muscle cells are esophageal smooth muscle cells, stomachic smooth muscle cells, smooth muscle cells of the small intestine, or smooth muscle cells of the large intestine.

21. A method of providing a subject with an amount of the protein of claim 1 effective to inhibit activation by CD40 ligand of cells bearing CD40 on the cell surface in the subject, comprising:

introducing into CD40-bearing cells of the subject, a nucleic acid sequence encoding the protein of claim 1, under conditions such that the cells express in the subject an activation inhibiting effective amount of the protein.

22. The method of claim 21, wherein the introducing of the nucleic acid into cells of the subject comprises:

a) treating cells of the subject ex vivo to insert the nucleic acid sequence into the cells; and

b) introducing the cells from step a) into the subject.

23. The method of claim 22, wherein the subject is a

mammal.

24. The method of claim 23, wherein the mammalian subject is a human.

25. The method of claim 21, wherein the CD40-bearing cells are selected from the group consisting of B cells, fibroblasts, endothelial cells, epithelial cells, T cells, basophils, macrophages, Reed-Steinberg cells, dendritic cells, renal cells, and smooth muscle cells.

26. The method of claim 25, wherein the B cells are resting B cells, primed B cells, myeloma cells, lymphocytic leukemia B cells, or B lymphoma cells.

27. The method of claim 21, wherein the epithelial cells are keratinocytes.

28. The method of claim 21, wherein the fibroblasts are synovial membrane fibroblasts, dermal fibroblasts, pulmonary fibroblasts, or liver fibroblasts.

29. The method of claim 21, wherein the renal cells are selected from the group consisting of glomerular endothelial cells, mesangial cells, distal tubule cells, proximal tubule cells, parietal epithelial cells, visceral epithelial cells, cells of a Henle limb, and interstitial inflammatory cells.

30. The method of claim 29, wherein the parietal epithelial cells are crescent parietal epithelial cells.

31. The method of claim 21, wherein the smooth muscle cells are smooth muscle cells of the bladder, vascular smooth muscle cells, aortic smooth muscle

cells, coronary smooth muscle cells, pulmonary smooth muscle cells, or gastrointestinal smooth muscle cells.

32. The method of claim 31, wherein the gastrointestinal smooth muscle cells are esophageal smooth muscle cells, stomachic smooth muscle cells, smooth muscle cells of the small intestine, or smooth muscle cells of the large intestine.

33. A method of treating a condition characterized by an aberrant or unwanted level of CD40-mediated intracellular signaling, in a subject, comprising providing the subject with a therapeutically effective amount of an agent capable of inhibiting CD40-mediated intracellular signaling in cells bearing CD40 on the cell surface.

34. The method of claim 33, wherein the agent is the protein of claim 1.

35. The method of claim 34, wherein the protein is provided by introducing into CD40-bearing cells of the subject, a nucleic acid sequence encoding the protein under conditions such that the cells express the protein according to the method of claim 21.

36. The method of claim 33, wherein the agent is a small molecule.

37. The method of claim 33, wherein the molecule is modified from a lead inhibitory agent.

38. The method of claim 33, wherein the condition is organ rejection in a subject receiving transplant organs, or an immune response in a subject

receiving gene therapy.

39. The method of claim 38, wherein the transplant organ is a kidney, heart or liver.

40. The method of claim 33, wherein the condition is a CD40-dependent immune response.

41. The method of claim 40, wherein the CD40-dependent immune response is an autoimmune response in a subject suffering from an autoimmune disease.

42. The method of claim 41, wherein the autoimmune disease comprises rheumatoid arthritis, Myasthenia gravis, systemic lupus erythematosus, Graves' disease, idiopathic thrombocytopenia purpura, hemolytic anemia, diabetes mellitus, a drug-induced autoimmune disease, psoriasis, or hyper IgE syndrome.

43. A method of claim 42, wherein the drug-induced autoimmune disease is drug-induced lupus.

44. The method of claim 40, wherein the immune response comprises induction of CD23, CD80 upregulation, rescue from CD95-mediated apoptosis, rescue from apoptosis in a subject undergoing chemotherapy against a tumor, or autoimmune manifestations of an infectious disease.

45. The method of claim 44, wherein the autoimmune manifestations are derived from Reiter's syndrome, spondyloarthritis, Lyme disease, HIV infections, syphilis or tuberculosis.

46. The method of claim 33, wherein the condition is an allergic response.

47. A method of claim 46, wherein the allergic response is hay fever or a penicillin allergy.

48. The method of claim 33, wherein the condition is dependent on CD40 ligand-induced activation of fibroblast cells.

49. The method of claim 48, wherein the condition is selected from the group consisting of arthritis, scleroderma, and fibrosis.

50. The method of claim 49, wherein the arthritis is rheumatoid arthritis, non-rheumatoid inflammatory arthritis, arthritis associated with Lyme disease, or osteoarthritis.

51. The method of claim 49, wherein the fibrosis is pulmonary fibrosis, hypersensitivity pulmonary fibrosis, or a pneumoconiosis.

52. The method of claim 51, wherein the pulmonary fibrosis is pulmonary fibrosis secondary to adult respiratory distress syndrome, drug-induced pulmonary fibrosis, idiopathic pulmonary fibrosis, or hypersensitivity pneumonitis.

53. The method of claim 51, wherein the pneumoconiosis is asbestosis, siliconosis, or Farmer's lung.

54. The method of claim 49, wherein the fibrosis is a fibrotic disease of the liver or lung.

55. The method of claim 54, wherein the fibrotic disease of the lung is caused by rheumatoid arthritis or scleroderma.

56. The method of claim 54, wherein the fibrotic

disease of the liver is selected from the group consisting of:

Hepatitis-C;
Hepatitis-B;
cirrhosis;
cirrhosis of the liver secondary to a toxic insult;
cirrhosis of the liver secondary to drugs;
cirrhosis of the liver secondary to a viral infection; and
cirrhosis of the liver secondary to an autoimmune disease.

57. The method of claim 56, wherein the toxic insult is alcohol consumption.

58. The method of claim 56, wherein the viral infection is Hepatitis B, Hepatitis C, or hepatitis non-B non-C.

59. The method of claim 56, wherein the autoimmune disease is primary biliary cirrhosis, or Lupoid hepatitis.

60. The method of claim 33, wherein the condition is dependent on CD40 ligand-induced activation of endothelial cells.

61. The method of claim 60, wherein the condition is selected from the group consisting of atherosclerosis, reperfusion injury, allograft rejection, organ rejection, and chronic inflammatory autoimmune diseases.

62. The method of claim 61, wherein the atherosclerosis is accelerated atherosclerosis associated with organ transplantation.

63. The method of claim 61, wherein the chronic inflammatory autoimmune disease is vasculitis, rheumatoid arthritis, scleroderma, or multiple sclerosis.

64. The method of claim 33, wherein the condition is dependent on CD40 ligand-induced activation of epithelial cells.

65. The method of claim 64 wherein the epithelial cells are keratinocytes, and the condition is psoriasis.

66. The method of claim 33, wherein the condition is an inflammatory kidney disease.

67. The method of claim 66, wherein the inflammatory kidney disease is not initiated by autoantibody deposition in kidney.

68. The method of claim 66, wherein the kidney disease is selected from the group consisting of:
membranous glomerulonephritis;
minimal change disease/acute tubular necrosis;
pauci-immune glomerulonephritis;
focal segmental glomerulosclerosis;
interstitial nephritis;
antitissue antibody-induced glomerular injury;
circulating immune-complex disease;
a glomerulopathy associated with a multisystem disease; and
drug-induced glomerular disease.

69. The method of claim 68, wherein the antitissue antibody-induced glomerular injury is anti-basement membrane antibody disease.

70. The method of claim 68, wherein the circulating

immune-complex disease is selected from the group consisting of:

infective endocarditis;
leprosy;
syphilis;
hepatitis B;
malaria; and
a disease associated with an endogenous antigen.

71. The method of claim 70, wherein the endogenous antigen is DNA, thyroglobulin, an autologous immunoglobulin, erythrocyte stroma, a renal tubule antigen, a tumor-specific antigen, or a tumor-associated antigen.

72. The method of claim 68 wherein the glomerulopathy associated with a multisystem disease is selected from the group consisting of:

diabetic nephropathy;
systemic lupus erythematosus;
Goodpasture's disease;
Henoch-Schönlein purpura;
polyarteritis;
Wegener's granulomatosis;
cryoimmunoglobulinemia;
multiple myeloma;
Waldenström's macroglobulinemia; and
amyloidosis.

73. The method of claim 68, wherein the pauci-immune glomerulonephritis is ANCA+ pauci-immune glomerulonephritis, or Wegener's granulomatosis.

74. The method of claim 68, wherein the interstitial nephritis is drug-induced interstitial nephritis.

75. The method of claim 66 wherein the kidney disease affects renal tubules.

76. The method of claim 75, wherein the kidney disease which affects renal tubules is selected from the group consisting of:

a kidney disease associated with a toxin;
a neoplasia;
hypersensitivity nephropathy;
Sjögren's syndrome; and
AIDS.

77. The method of claim 33, wherein the condition is a smooth muscle cell-dependent disease.

78. The method of claim 77, wherein the smooth muscle cell-dependent disease is a vascular disease.

79. The method of claim 78, wherein the vascular disease is atherosclerosis.

80. The method of claim 77, wherein the smooth muscle cell-dependent disease is a gastrointestinal disease.

81. The method of claim 80, wherein the gastrointestinal disease is selected from the group consisting of: esophageal dysmotility, inflammatory bowel disease, and scleroderma.

82. The method of claim 77, wherein the smooth muscle cell-dependent disease is a bladder disease.

83. The method of claim 33, wherein the condition is associated with Epstein-Barr virus.

84. The method of claim 83, wherein the condition is

selected from the group consisting of mononucleosis, B cell tumors, Burkitt's lymphoma, and nasopharyngeal carcinoma.

85. An isolated nucleic acid molecule encoding the protein of claim 1.

86. The nucleic acid molecule of claim 85, wherein the molecule is DNA.

87. A vector comprising the nucleic acid molecule of claim 85 operably linked to a transcriptional control sequence recognized by a host cell transformed with the vector.

88. The vector of claim 87, wherein the vector is a plasmid.

89. A method of identifying an agent capable of inhibiting CD40-mediated intracellular signaling in a cell expressing CD40 on the cell surface, comprising providing the cell with the agent under conditions permitting activation of the cell in the absence of the agent, and determining decreased or absent activation, thereby identifying an agent capable of inhibiting CD40-mediated intracellular signaling in a cell expressing CD40 on the cell surface.

90. The method of claim 89, wherein the activation comprises up-regulation of CD23.

91. The method of claim 89, wherein the conditions permitting activation of the cell comprises contacting the cell with CD40 ligand or portion thereof effective to activate the cell.

TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

Abstract of the Disclosure

Overexpression of a CRAF1 (CD40 receptor-associated factor 1) gene truncated by 323 to about 414 amino acids at the amino inhibits CD40-mediated cell activation, and is used to treat conditions characterized by an unwanted level of CD40-mediated intracellular signaling.

Applicant or Patentee: David Baltimore et al. Attorney's
Serial or Patent No.: Not Yet Known Docket No: 50659
Filed or Issued: Herewith
Title of Invention or Patent: TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

67477 U.S. PTO
03/10/97

VERIFIED STATEMENT (DECLARATION) CLAIMING
SMALL ENTITY STATUS UNDER 37 C.F.R. §1.9(f)
AND §1.27(d) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

Name of Organization: The Trustees of Columbia University in the City of New York

Address of Organization: 110 Low Memorial Library, West 116th Street & Broadway
New York, New York 10027

TYPE OF ORGANIZATION:

X UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
____ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE 26 U.S.C. §§501(a) and 501(c)(3)
____ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
NAME OF STATE: _____
CITATION OF STATUTE: _____
____ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE 26 U.S.C. §§501(a) and 501(c)(3) IF LOCATED IN THE UNITED STATES OF AMERICA
____ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
NAME OF STATE: _____
CITATION OF STATUTE: _____

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. §1.9(e)* for purposes of paying reduced fees under 35 U.S.C. §41(a) and 41(b), with regard to the invention entitled TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

by inventor(s) David Baltimore, Genhong Cheng, Aileen Cleary, Seth Lederman and Zheng-sheng Ye described in:

X the specification filed herewith
____ application serial no. _____ filed _____
____ patent no. _____ issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive each individual, concern, or organization known to have rights to the invention is listed below[a] and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. §1.9(d)* or a nonprofit organization under 37 C.F.R. 1.9(e)*

[a]NOTE: Separate verified statements are required from each person, concern, or organization having rights to the invention averring to their status as small entities. 37 C.F.R. §1.27.

Name: None
Address: _____

____ Individual ____ Small Business Concern ____ Nonprofit Organization

*See Reverse

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. 37 C.F.R. §1.28(b)*.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

Name of Person Signing: Mr. Jack M. Granowitz
Title In Organization: Executive Director, Columbia Innovation Enterprise
Address: Columbia University, Engineering Terrace - Suite 363
West 120th Street and Amsterdam, New York, New York 10027
Signature: Jack M. Granowitz
Date Of Signature: March 7, 1997

*See Reverse

#3

DECLARATION AND POWER OF ATTORNEY

As a below-named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

the specification of which:
(check one)

___ *is attached hereto*

X *was filed on* March 10, 1997 *as*

Application Serial No. 08/813,323

and was amended _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 (a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International Application which designated at least one country other than the United States, listed below. I have also identified below any foreign application for patent or inventor's certificate, or PCT International Application having a filing date before that of the earliest application from which priority is claimed:

Prior Foreign Application(s)			*Priority Claimed*	
Number	*Country*	*Filing Date*	*Yes*	*No*
N/A			___	___
			___	___
			___	___
			___	___
			___	___
			___	___

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below

Provisional Application No	*Filing Date*	*Status*
60/013,199	March 11, 1996	Pending

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States Application(s), or Section 365(c) of any PCT International Application(s) designating the United States listed below. Insofar as this application discloses and claims subject matter in addition to that disclosed in any such prior Application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56, which became available between the filing date(s) of such prior Application(s) and the national or PCT international filing date of this application

Application Serial No	*Filing Date*	*Status*
None		

And I hereby appoint

John P. White (Reg. No. 28,678); Thomas F. Moran (Reg. No. 16,579); Norman H. Zivin (Reg. No. 25,385); Ivan S. Kavrukov (Reg. No. 25,161); Christopher C. Dunham (Reg. No. 22,031); Thomas G. Caruso (Reg. No. 30,616); Robert D. Katz (Reg. No. 30,141); Peter J. Phillips (Reg. No. 29,691); Richard S. Milner (Reg. No. 33,970); Albert Wai-Kit Chan (Reg. No. 36,479); Mary Anne P. Tanner (Reg. No. 40,197); and Mary Catherine DiNunzio (Reg. No. 37,306)

and each of them, all c o Cooper & Dunham LLP, 1185 Avenue of the Americas, New York, New York 10036, my attorneys, each with full power of substitution and revocation, to prosecute this application, to make alterations and amendments therein, to receive the patent, to transact all business in the Patent and Trademark Office connected therewith and to file any International Applications which are based thereon under the provisions of the Patent Cooperation Treaty.

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White, Esq. *Reg No* 28,678
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor David Baltimore

Inventor's signature

Citizenship United States of America *Date of signature* 16 July 97

Residence 508 Union Wharf, Boston, Massachusetts 02109

Post Office Address same as residence address

Full name of joint inventor (if any) Genhong Cheng

Inventor's signature

Citizenship People's Republic of China *Date of signature*

Residence 827 Leverin Avenue, Apt. 304, Los Angeles, California 90024

Post Office Address same as residence address

Full name of joint inventor (if any) Zheng-Sheng Ye

Inventor's signature

Citizenship United States of America *Date of signature*

Residence 1233 York Avenue, New York, New York 10021

Post Office Address same as residence address

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White, Esq. *Reg No* 28,678
Cooper & Dunham LLP
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New York, New York 10036
Tel (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor David Baltimore

Inventor's signature __________

Citizenship United States of America *Date of signature* __________

Residence 508 Union Wharf, Boston, Massachusetts 02109

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Full name of joint inventor (if any) Genhong Cheng

Inventor's signature [signature]

Citizenship People's Republic of China *Date of signature* 7/10/97

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Full name of joint inventor (if any) Zheng-Sheng Ye

Inventor's signature __________

Citizenship United States of America *Date of signature* __________

Residence 1233 York Avenue, New York, New York 10021

Post Office Address same as residence address

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true: and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor David Baltimore

Inventor's signature ______

Citizenship United States of America *Date of signature* ______

Residence 508 Union Wharf, Boston, Massachusetts 02109

Post Office Address same as residence address

Full name of joint inventor (if any) Genhong Cheng

Inventor's signature ______

Citizenship People's Republic of China *Date of signature* ______

Residence 827 Leverin Avenue, Apt. 304, Los Angeles, California 90024

Post Office Address same as residence address

Full name of joint inventor (if any) Zheng-Sheng Ye

Inventor's signature [signature]

Citizenship United States of America *Date of signature* ______

Residence 1233 York Avenue, New York, New York 10021

Post Office Address same as residence address

Please address all communications, and direct all telephone calls, regarding this application to:

John P. White, Esq. *Reg. No.* 28,678
Cooper & Dunham LLP
1185 Avenue of the Americas
New York, New York 10036
Tel. (212) 278-0400

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first joint inventor Seth Lederman

Inventor's signature Seth Lederman

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Full name of joint inventor (if any) Aileen Cleary

Inventor's signature Aileen Cleary

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#24

Dkt. 0575/50659/JPW/JML

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : David Baltimore et al.

Serial No. : 08/813,323

Filed : March 10, 1997 Art Unit: 1815

For : TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

1185 Avenue of the Americas
New York, New York 10036
July 30, 1997

Assistant Commissioner for Patents
Washington, D.C. 20231

BOX: Application Processing Division
Special Processing and Correspondence Branch

Sir:

AMENDMENT IN RESPONSE TO MAY 30, 1997 NOTICE TO FILE MISSING PARTS OF APPLICATION AND NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

This Amendment is submitted in response to a Notice to File Missing Parts of Application under 37 C.F.R. § 1.53(d) and Notice to Comply with Requirements For Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures which was issued by the United States Patent and Trademark Office in connection with the above-identified patent application on May 30, 1997. A response to the May 30, 1997 Notice is due July 30, 1997. Accordingly, this Amendment is timely filed.

Please amend the subject application as follows:

In the specification:

Please insert the Sequence Listing set forth as new pages 40-48, attached hereto as Exhibit C, after page 39 of the subject application; and

Please renumber originally filed pages 40-52 as new pages 49-61.

REMARKS

Claims 1-20 are pending.

Applicants attach hereto a copy of the Notice as **Exhibit A**. Applicants hereby submit an executed Declaration and Power of Attorney pursuant to 37 C.F.R. § 1.53(d) and in compliance with 37 C.F.R. § 1.63 (**Exhibit B** hereto). The Declaration refers to the application's above-identified serial number and filing date.

The surcharge for responding to the Notice to File Missing Parts of Application under 37 C.F.R. § 1.53(d) is SIXTY-FIVE DOLLARS ($65.00) for a small entity. Applicants previously established small-entity status and such status is still applicable. A check for this amount ($65.00) is enclosed.

Applicants also submit herewith a Sequence Listing attached hereto as **Exhibit C** in compliance with the requirements of 37 C.F.R. §1.824. In addition, applicants submit herewith the Sequence Listing on the enclosed computer diskette, which has the same content as the paper copy attached as **Exhibit C**. Applicants submit as **Exhibit D**, a Statement in accordance with 37 C.F.R. §1.821(f) certifying that the computer readable form containing the nucleic acid and/or amino acid sequences required by 37 C.F.R. §1.821(f) and submitted in connection with the above-identified application, has the same information which is submitted in this amendment to subject application under the section entitled "Sequence Listing" (**Exhibit C**).

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorney invites the Examiner to telephone him at the number

provided below.

No fee, other than the $65.00 surcharge fee, is deemed necessary in connection with the filing of this Amendment. If any such fee is required, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 03-3125.

Respectfully submitted,

Albert Wai-Kit Chan

John P. White
Registration No. 28,678
Albert Wai-Kit Chan
Registration No. 36,479
Attorneys for Applicants
Cooper & Dunham LLP
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I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to:
Assistant Commissioner for Patents
Washington, D.C. 20231.

Albert Wai-Kit Chan 7/30/97
Albert Wai-Kit Chan Date
Reg. No. 36,479

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Baltimore, David
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Cleary, Aileen
Lederman, Seth
Ye, Zheng-sheng

(ii) TITLE OF INVENTION: TRUNCATED CRAF1 INHIBITS CD40 SIGNALING

(iii) NUMBER OF SEQUENCES: 5

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(E) COUNTRY: USA
(F) ZIP: 10036

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: White, John P
(B) REGISTRATION NUMBER: 28,678
(C) REFERENCE/DOCKET NUMBER: 50659

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 566 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Met Glu Ser Ser Lys Lys Met Asp Ala Ala Gly Thr Leu Gln Pro Asn
1               5                  10                  15

Pro Pro Leu Lys Leu Gln Pro Asp Arg Gly Ala Gly Ser Val Leu Val
            20                  25                  30

Pro Glu Gln Gly Gly Tyr Lys Glu Lys Phe Val Lys Thr Val Glu Asp
        35                  40                  45

Lys Tyr Lys Cys Glu Lys Cys Arg Leu Val Leu Cys Asn Pro Lys Gln
    50                  55                  60

Thr Glu Cys Gly His Arg Phe Cys Glu Ser Cys Met Ala Ala Leu Leu
65                  70                  75                  80

Ser Ser Ser Ser Pro Lys Cys Thr Ala Cys Gln Glu Ser Ile Ile Lys
                85                  90                  95

Asp Lys Val Phe Lys Asp Asn Cys Cys Lys Arg Glu Ile Leu Ala Leu
            100                 105                 110

Gln Val Tyr Cys Arg Asn Glu Gly Arg Gly Cys Ala Glu Gln Leu Thr
        115                 120                 125

Leu Gly His Leu Leu Val His Leu Lys Asn Glu Cys Gln Phe Glu Glu
    130                 135                 140

Leu Pro Cys Leu Arg Ala Asp Cys Lys Glu Lys Val Leu Arg Lys Asp
145                 150                 155                 160

Leu Arg Asp His Val Glu Lys Ala Cys Lys Tyr Arg Glu Ala Thr Cys
                165                 170                 175

Ser His Cys Lys Ser Gln Val Pro Met Ile Lys Leu Gln Lys His Glu
            180                 185                 190

Asp Thr Asp Cys Pro Cys Val Val Val Ser Cys Pro His Lys Cys Ser
        195                 200                 205

Val Gln Thr Leu Leu Arg Ser Glu Leu Ser Ala His Leu Ser Glu Cys
    210                 215                 220

Val Asn Ala Pro Ser Thr Cys Ser Phe Lys Arg Tyr Gly Cys Val Phe
225                 230                 235                 240

Gln Gly Thr Asn Gln Gln Ile Lys Ala His Glu Ala Ser Ser Ala Val
                245                 250                 255

Gln His Val Asn Leu Leu Lys Glu Trp Ser Asn Ser Leu Glu Lys Lys
            260                 265                 270

Val Ser Leu Leu Gln Asn Glu Ser Val Glu Lys Asn Lys Ser Ile Gln
        275                 280                 285

Ser Leu His Asn Gln Ile Cys Ser Phe Glu Ile Glu Ile Glu Arg Gln
```

```
    290                 295                 300

Lys Glu Met Leu Arg Asn Asn Glu Ser Lys Ile Leu His Leu Gln Arg
305                 310                 315                 320

Val Ile Asp Ser Gln Ala Glu Lys Leu Lys Glu Leu Asp Lys Glu Ile
                325                 330                 335

Arg Pro Phe Arg Gln Asn Trp Glu Glu Ala Asp Ser Met Lys Ser Ser
            340                 345                 350

Val Glu Ser Leu Gln Asn Arg Val Thr Glu Leu Glu Ser Val Asp Lys
        355                 360                 365

Ser Ala Gly Gln Ala Ala Arg Asn Thr Gly Leu Leu Glu Ser Gln Leu
    370                 375                 380

Ser Arg His Asp Gln Thr Leu Ser Val His Asp Ile Arg Leu Ala Asp
385                 390                 395                 400

Met Asp Leu Arg Phe Gln Val Leu Glu Thr Ala Ser Tyr Asn Gly Val
                405                 410                 415

Leu Ile Trp Lys Ile Arg Asp Tyr Lys Arg Arg Lys Gln Glu Ala Val
            420                 425                 430

Met Gly Lys Thr Leu Ser Leu Tyr Ser Gln Pro Phe Tyr Thr Gly Tyr
        435                 440                 445

Phe Gly Tyr Lys Met Cys Ala Arg Val Tyr Leu Asn Gly Asp Gly Met
    450                 455                 460

Gly Lys Gly Thr His Leu Ser Leu Phe Phe Val Ile Met Arg Gly Glu
465                 470                 475                 480

Tyr Asp Ala Leu Leu Pro Trp Pro Phe Lys Gln Lys Val Thr Leu Met
                485                 490                 495

Leu Met Asp Gln Gly Ser Ser Arg Arg His Leu Gly Asp Ala Phe Lys
            500                 505                 510

Pro Asp Pro Asn Ser Ser Ser Phe Lys Lys Pro Thr Gly Glu Met Asn
        515                 520                 525

Ile Ala Ser Gly Cys Pro Val Phe Val Ala Gln Thr Val Leu Glu Asn
    530                 535                 540

Gly Thr Tyr Ile Lys Asp Asp Thr Ile Phe Ile Lys Val Ile Val Asp
545                 550                 555                 560

Thr Ser Asp Leu Pro Asp
                565
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 568 amino acids
(B) TYPE: amino acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..568

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Glu Ser Ser Lys Lys Met Asp Ser Pro Gly Ala Leu Gln Thr Asn
1               5                   10                  15

Pro Pro Leu Lys Leu His Thr Asp Arg Ser Ala Gly Thr Pro Val Phe
            20                  25                  30

Val Pro Glu Gln Gly Gly Tyr Lys Glu Lys Phe Val Lys Thr Val Glu
        35                  40                  45

Asp Lys Tyr Lys Cys Glu Lys Cys His Leu Val Leu Cys Ser Pro Lys
    50                  55                  60

Gln Thr Glu Cys Gly His Arg Phe Cys Glu Ser Cys Met Ala Ala Leu
65                  70                  75                  80

Leu Ser Ser Ser Ser Pro Lys Cys Thr Ala Cys Gln Glu Ser Ile Val
                85                  90                  95

Lys Asp Lys Val Phe Lys Asp Asn Cys Cys Lys Arg Glu Ile Leu Ala
            100                 105                 110

Leu Gln Ile Tyr Cys Arg Asn Glu Ser Arg Gly Cys Ala Glu Gln Leu
        115                 120                 125

Thr Leu Gly His Leu Leu Val His Leu Lys Asn Asp Cys His Phe Glu
    130                 135                 140

Glu Leu Pro Cys Val Arg Pro Asp Cys Lys Glu Lys Val Leu Arg Lys
145                 150                 155                 160

Asp Leu Arg Asp His Val Glu Lys Ala Cys Lys Tyr Arg Glu Ala Thr
                165                 170                 175

Cys Ser His Cys Lys Ser Gln Val Pro Met Ile Ala Leu Gln Lys His
            180                 185                 190

Glu Asp Thr Asp Cys Pro Cys Val Val Val Ser Cys Pro His Lys Cys
        195                 200                 205

Ser Val Gln Thr Leu Leu Arg Ser Glu Leu Ser Ala His Leu Ser Glu
    210                 215                 220

Cys Val Asn Ala Pro Ser Thr Cys Ser Phe Lys Arg Tyr Gly Cys Val
225                 230                 235                 240
```

```
Phe Gln Gly Thr Asn Gln Gln Ile Lys Ala His Glu Ala Ser Ser Ala
                245                 250                 255

Val Gln His Val Asn Leu Leu Lys Glu Trp Ser Asn Ser Leu Glu Lys
            260                 265                 270

Lys Val Ser Leu Leu Gln Asn Glu Ser Val Glu Lys Asn Lys Ser Ile
        275                 280                 285

Gln Ser Leu His Asn Gln Ile Cys Ser Phe Glu Ile Glu Ile Glu Arg
    290                 295                 300

Gln Lys Glu Met Leu Arg Asn Asn Glu Ser Lys Ile Leu His Leu Gln
305                 310                 315                 320

Arg Val Ile Asp Ser Gln Ala Glu Lys Leu Lys Glu Leu Asp Lys Glu
                325                 330                 335

Ile Arg Pro Phe Arg Gln Asn Trp Glu Glu Ala Asp Ser Met Lys Ser
            340                 345                 350

Ser Val Glu Ser Leu Gln Asn Arg Val Thr Glu Leu Glu Ser Val Asp
        355                 360                 365

Lys Ser Ala Gly Gln Val Ala Arg Asn Thr Gly Leu Leu Glu Ser Gln
    370                 375                 380

Leu Ser Arg His Asp Gln Met Leu Ser Val His Asp Ile Arg Leu Ala
385                 390                 395                 400

Asp Met Asp Leu Arg Phe Gln Val Leu Glu Thr Ala Ser Tyr Asn Gly
                405                 410                 415

Val Leu Ile Trp Lys Ile Arg Asp Tyr Lys Arg Arg Lys Gln Glu Ala
            420                 425                 430

Val Met Gly Lys Thr Leu Ser Leu Tyr Ser Gln Pro Phe Tyr Thr Gly
        435                 440                 445

Tyr Phe Gly Tyr Lys Met Cys Ala Arg Val Tyr Leu Asn Gly Asp Gly
    450                 455                 460

Met Gly Lys Gly Thr His Leu Ser Leu Phe Phe Val Ile Met Arg Gly
465                 470                 475                 480

Glu Tyr Asp Ala Leu Leu Pro Trp Pro Phe Lys Gln Lys Val Thr Leu
                485                 490                 495

Met Leu Met Asp Gln Gly Ser Ser Arg Arg His Leu Gly Asp Ala Phe
            500                 505                 510

Lys Pro Asp Pro Asn Ser Ser Ser Phe Lys Lys Pro Thr Gly Glu Met
        515                 520                 525

Asn Ile Ala Ser Gly Cys Pro Val Phe Val Ala Gln Thr Val Leu Glu
    530                 535                 540

Asn Gly Thr Tyr Ile Lys Asp Asp Thr Ile Phe Ile Lys Val Ile Val
```

545 550 555 560

Asp Thr Ser Asp Leu Pro Asp Pro
565

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2359 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCGGCGGAG GATGCGCGCG GCGCCTGAGC CGGCCGAACG GGCGGCCTCG GGGTACAGGG 60

TCCCCATTAC TTGAAGGATA AGGCTGGCAC GGCTCCGACG TCTGTGTGGA AGCTTCTCCC 120

TCCCTTCTGA GCTTCTCTAG ACTCCTTACA GCGCACGGCA CAGAATTTCA GTTTCCTAAG 180

ATGGAGTCAA GCAAAAAGAT GGATGCTGCT GGCACACTGC AGCCTAACCC ACCCCTAAAG 240

CTGCAGCCTG ATCGCGGCGC AGGGTCCGTG CTCGTGCCGG AGCAAGGAGG CTACAAGGAG 300

AAGTTTGTGA AGACGGTGGA AGACAAGTAC AAGTGCGAGA AGTGCCGCCT GGTGCTGTGC 360

AACCCGAAGC AGACGGAGTG TGGCCACCGG TTCTGCGAGA GCTGCATGGC CGCCCTGCTG 420

AGCTCCTCCA GTCCAAAATG CACAGCGTGC CAAGAAAGCA TCATCAAAGA CAAGGTGTTT 480

AAGGATAATT GCTGCAAGAG AGAGATTCTG GCCCTTCAGG TCTACTGTCG GAATGAAGGC 540

AGAGGTTGTG CGGAGCAGCT GACTCTGGGA CATCTGCTGG TGCACCTAAA AAATGAATGT 600

CAGTTTGAGG AACTTCCCTG TCTGCGTGCC GACTGCAAAG AAAAAGTACT GAGAAAAGAC 660

TTGCGGGATC ACGTGGAAAA GGCCTGTAAA TACCGCGAGG CCACGTGCAG TCACTGCAAG 720

AGCCAAGTGC CCATGATCAA ACTGCAGAAA CATGAAGACA CAGATTGTCC CTGTGTGGTG 780

GTATCCTGCC CTCACAAGTG CAGCGTTCAG ACTCTTCTAA GGAGTGAGTT GAGTGCACAC 840

TTGTCCGAGT GTGTCAATGC CCCCAGCACC TGTAGTTTTA AGCGCTATGG CTGCGTTTTT 900

CAGGGTACAA ACCAGCAGAT CAAGGCCCAT GAGGCCAGCT CCGCGGTACA GCACGTGAAC 960

CTGCTGAAGG AGTGGAGCAA CTCCCTGGAG AAGAAGGTTT CCCTGCTGCA GAATGAAAGT 1020

GTTGAGAAAA ACAAGAGCAT CCAAAGCCTG CACAACCAGA TCTGCAGCTT TGAGATCGAG 1080

ATTGAGAGGC AGAAGGAGAT GCTCCGAAAC AACGAGTCCA AGATCCTTCA CCTGCAGCGG 1140

GTAATCGACA GCCAAGCAGA GAAACTGAAA GAACTGGACA AGGAGATCCG TCCCTTCCGG 1200

CAGAACTGGG AGGAAGCGGA CAGCATGAAG AGCAGTGTGG AGTCCCTCCA GAACCGAGTG 1260

ACTGAGCTGG AGAGCGTAGA CAAAAGTGCG GGGCAGGCGG CTCGCAACAC AGGCTTGCTG 1320

GAGTCCCAGC TGAGCCGGCA TGACCAGACG TTGAGTGTTC ATGACATCCG CTTGGCCGAC 1380

ATGGACCTGC GGTTCCAGGT CCTCGAGACC GCCAGCTACA ACGGGGTGCT GATCTGGAAG 1440

ATCCGTGACT ACAAGCGCCG GAAGCAGGAG GCCGTCATGG GGAAGACCCT GTCTCTCTAC 1500

AGCCAGCCTT TCTACACAGG TTATTTTGGC TATAAGATGT GTGCCAGGGT CTACCTGAAT 1560

GGGGACGGAA TGGGGAAAGG GACACACTTG TCGCTGTTTT TTGTCATTAT GCGTGGAGAA 1620

TATGATGCTC TGTTGCCATG GCCGTTCAAG CAGAAAGTGA CACTTATGCT GATGGATCAG 1680

GGGTCCTCTC GCCGTCATCT GGGAGATGCG TTCAAGCCTG ACCCCAACAG CAGCAGCTTC 1740

AAGAAACCCA CCGGAGAGAT GAATATCGCC TCTGGCTGCC CAGTCTTTGT CGCCCAAACT 1800

GTTCTAGAGA ACGGGACGTA TATTAAAGAT GATACAATCT TTATTAAGGT CATAGTGGAT 1860

ACCTCGGATC TGCCTGACCC CTGACAAGAA AGCAGGGCGG TGGATTCAGC AGAAGGTAAC 1920

TCCTCTGGGG GGGTGAGCTA GTGTCTTCAC GGAGGTCCTC GCCCTCAGAA AGGACCTTGT 1980

GGCGCAGAGG AAGCAGCCGG AGGAGGAGAA GGAGGTCGAG TGGCTGGCAG GAGAGCCACA 2040

TGTGAAAACA GACCCCAACG GATTTTCTAA TAAACTAGCC ACACCCACTC TGAAGGATTA 2100

TTTATCCATC AACAAGATAA ATACTGCTGT CAGAGAAGGT TTTCATTTTC ATTTTAAAAG 2160

ATCTAGTATT AAGGTGGGAA CATATATGCT AAAAAGAAAC ATGATTTTTC TTCCTTAACT 2220

TAAACACCAA AAAGAGAACA CATGTGGGGG TAGCTGGAGT GTGTACAGTA CCTCGAGGGC 2280

TTAAAATCAT AAACAATCAC ATACTCATCC TAAAATTCAG GGTGCAACTC CGTTTCAAAT 2340

ATTGTATATT GTCTATTTA 2359

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2455 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGGGGGAGCG CGGCGCGGCC GCCGCGTGCG CGAGCCGGGG TTGCAGCCCA GCCGGGACTT 60

TCCAGCCGGC GGCAGCCGCG GCGGTCGTCG GCTCTTCCCC GCCCCCCGTC ATGGGGCAGC 120

CCGGGGAGCA GAACGCTGCG GACCGCGGCG GAGGACGCGC CCGGCGCCCC TGAGCCGGCC 180

GAGCGGCGAC GGACCGCGAG AACTCCTCTT TCCTAAAATG GAGTCGAGTA AAAAGATGGA 240

CTCTCCTGGC GCGCTGCAGA CTAACCCGCC GCTAAAGCTG CACACTGACC GTAGTGCTGG 300

GACGCCAGTT TTTGTCCCTG AACAAGGAGG TTACAAGGAA AAGTTTGTGA AGACCGTGGA 360

GGACAAGTAC AAGTGTGAGA AGTGCCACCT GGTGCTGTGC AGCCCGAAGC AGACCGAGTG 420

TGGGCACCGC TTCTGCGAGA GCTGCATGGC GGCCCTGCTG AGCTCTTCAA GTCCAAAATG 480

TACAGCGTGT CAAGAGAGCA TCGTTAAAGA TAAGGTGTTT AAGGATAATT GCTGCAAGAG 540

AGAAATTCTG GCTCTTCAGA TCTATTGTCG GAATGAAAGC AGAGGTTGTG CAGAGCAGTT 600

AACGCTGGGA CATCTGCTGG TGCATTTAAA AAATGATTGC CATTTTGAAG AACTTCCATG 660

TGTGCGTCCT GACTGCAAAG AAAAGGTCTT GAGGAAAGAC CTGCGAGACC ACGTGGAGAA 720

GGCGTGTAAA TACCGGGAAG CCACATGCAG CCACTGCAAG AGTCAGGTTC CGATGATCGC 780

GCTGCAGAAA CACGAAGACA CCGACTGTCC CTGCGTGGTG GTGTCCTGCC CTCACAAGTG 840

CAGCGTCCAG ACTCTCCTGA GGAGCGAGTT GAGTGCACAC TTGTCAGAGT GTGTCAATGC 900

CCCCAGCACC TGTAGTTTTA AGCGCTATGG CTGCGTTTTT CAGGGGACAA ACCAGCAGAT 960

CAAGGCCCAC GAGGCCAGCT CCGCCGTGCA GCACGTCAAC CTGCTGAAGG AGTGGAGCAA 1020

CTCGCTCGAA AAGAAGGTTT CCTTGTTGCA GAATGAAAGT GTAGAAAAAA ACAAGAGCAT 1080

ACAAAGTTTG CACAATCAGA TATGTAGCTT TGAAATTGAA ATTGAGAGAC AAAAGGAAAT 1140

GCTTCGAAAT AATGAATCCA AAATCCTTCA TTTACAGCGA GTGATCGACA GCCAAGCAGA 1200

GAAACTGAAG GAGCTTGACA AGGAGATCCG GCCCTTCCGG CAGAACTGGG AGGAAGCAGA 1260

CAGCATGAAG AGCAGCGTGG AGTCCCTCCA GAACCGCGTG ACCGAGCTGG AGAGCGTGGA 1320

CAAGAGTGCG GGGCAAGTGG CTCGGAACAC AGGCCTGCTG GAGTCCCAGC TGAGCCGGCA 1380

TGACCAGATG CTGAGTGTGC ACGACATCCG CCTAGCCGAC ATGGACCTGC GCTTCCAGGT 1440

CCTGGAGACC GCCAGCTACA ATGGAGTGCT CATCTGGAAG ATTCGCGACT ACAAGCGGCG 1500

GAAGCAGGAG GCCGTCATGG GGAAGACCCT GTCCCTTTAC AGCCAGCCTT TCTACACTGG 1560

TTACTTTGGT TATAAGATGT GTGCCAGGGT CTACCTGAAC GGGGACGGGA TGGGGAAGGG 1620

GACGCACTTG TCGCTGTTTT TTGTCATCAT GCGTGGAGAA TATGATGCCC TGCTTCCTTG 1680

GCCGTTTAAG CAGAAAGTGA CACTCATGCT GATGGATCAG GGGTCCTCTC GACGTCATTT 1740

GGGAGATGCA TTCAAGCCCG ACCCCAACAG CAGCAGCTTC AAGAAGCCCA CTGGAGAGAT 1800

GAATATCGCC TCTGGCTGCC CAGTCTTTGT GGCCCAAACT GTTCTAGAAA ATGGGACATA 1860

TATTAAAGAT GATACAATTT TTATTAAAGT CATAGTGGAT ACTTCGGATC TGCCCGATCC 1920

CTGATAAGTA GCTGGGGAGG TGGATTTAGC AGAAGGCAAC TCCTCTGGGG GATTTGAACC 1980

GGTCTGTCTT CACTGAGGTC CTCGCGCTCA GAAAAGGACC TTGTGAGACG GAGGAAGCGG 2040

CAGAAGGCGG ACGCGTGCCG GCGGGAGGAG CCACGCGTGA GCACACCTGA CACGTTTTAT 2100

AATAGACTAG CCACACTTCA CTCTGAAGAA TTATTTATCC TTCAACAAGA TAAATATTGC 2160

TGTCAGAGAA GGTTTTCATT TTCATTTTTA AAGATCTAGT TAATTAAGGT GGAAAACATA 2220

TATGCTAAAC AAAAGAAACA TGATTTTTCT TCCTTAAACT TGAACACCAA AAAAACACAC 2280

ACACACACAC ACGTGGGGAT AGCTGGACAT GTCAGCATGT TAAGTAAAAG GAGAATTTAT 2340

GAAATAGTAA TGCAATTCTG ATATCTTCTT TCTAAAATTC AAGAGTGCAA TTTTGTTTCA 2400

AATACAGTAT ATTGTCTATT TTTAAGGCCT CCAAAAAAAA AAAAAATTCC GGCCG 2455

(2) INFORMATION FOR SEQ ID NO:5:

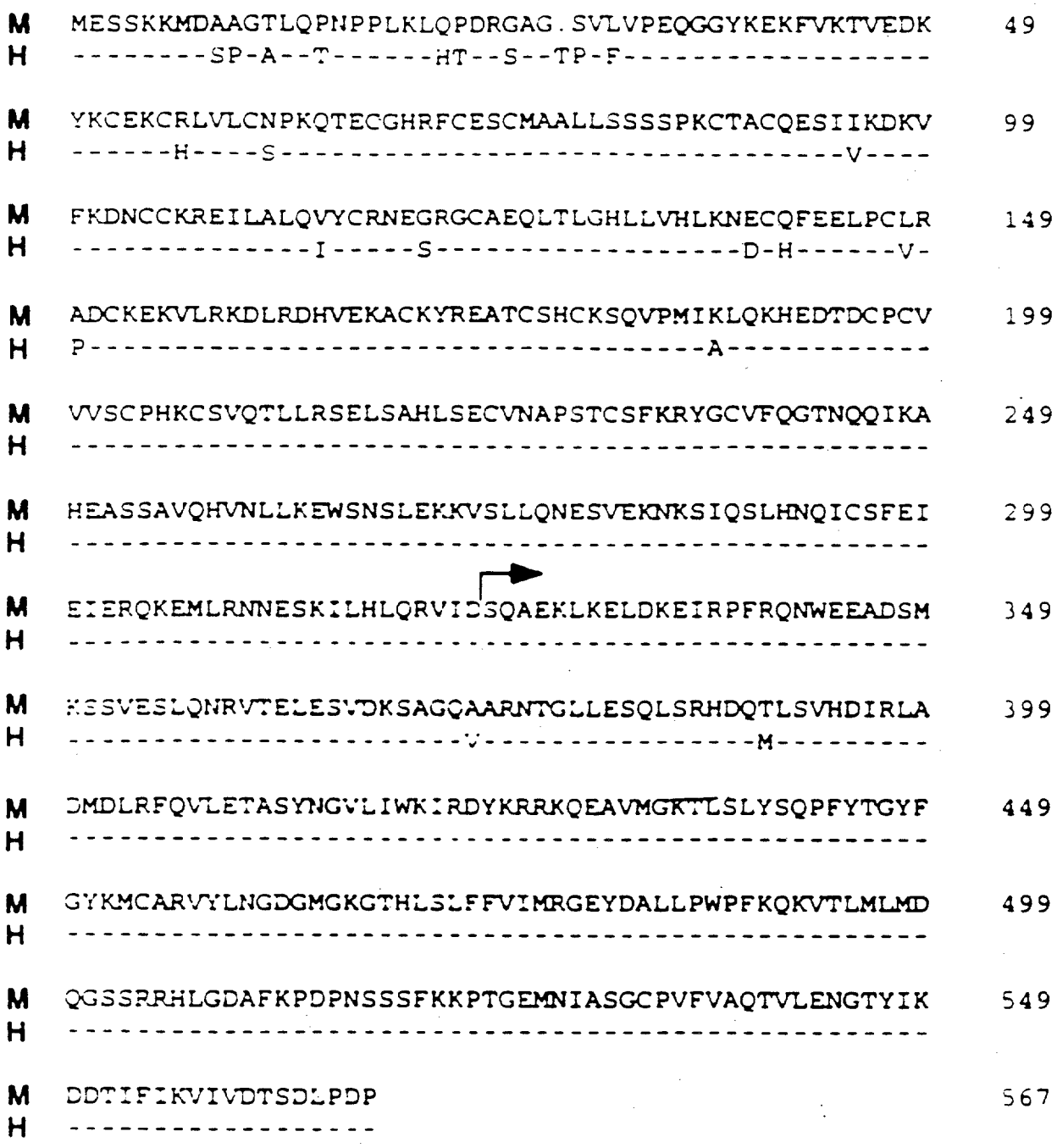
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Ala Cys Lys Tyr Arg
1 5

FIG. 1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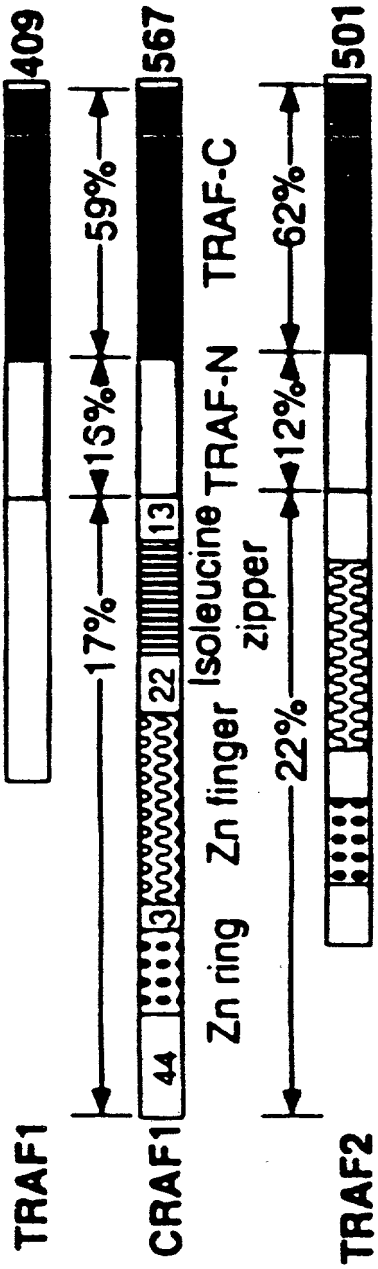
H --------SP-A--T------HT--S--TP-F----------
M YKCEKCRLVLCNPKQTECGHRFCESCMAALLSSSSPKCTACQESIIKDKV 99
H ------H----S----------V----
M FKDNCCKREILALQVYCRNEGRGCAEQLTLGHLLVHLKNECQFEELPCLR 149
H --------------I-----S----------D-H------V-
M ADCKEKVLRKDLRDHVEKACKYREATCSHCKSQVPMIKLQKHEDTDCPCV 199
H P----------A----------
M VVSCPHKCSVQTLLRSELSAHLSECVNAPSTCSFKRYGCVFQGTNQQIKA 249
H ----------
M HEASSAVQHVNLLKEWSNSLEKKVSLLQNESVEKNKSIQSLHNQICSFEI 299
H ----------
M EIERQKEMLRNNESKILHLQRVICSQAEKLKELDKEIRPFRQNWEEADSM 349
H ----------
M KESVESLQNRVTELESVDKSAGQAARNTGLLESQLSRHDQTLSVHDIRLA 399
H ----------V----------M---------
M DMDLRFQVLETASYNGVLIWKIRDYKRRKQEAVMGKTLSLYSQPFYTGYF 449
H ----------
M GYKMCARVYLNGDGMGKGTHLSLFFVIMRGEYDALLPWPFKQKVTLMLMD 499
H ----------
M QGSSRRHLGDAFKPDPNSSSFKKPTGEMNIASGCPVFVAQTVLENGTYIK 549
H ----------
M DDTIFIKVIVDTSDLPDP 567
H ----------

FIG. 2A


409
567
501
17%
15%
59%
22%
12%
62%
TRAF1
CRAF1
TRAF2
44
3
22
13
Zn ring
Zn finger
Isoleucine zipper
TRAF-N
TRAF-C

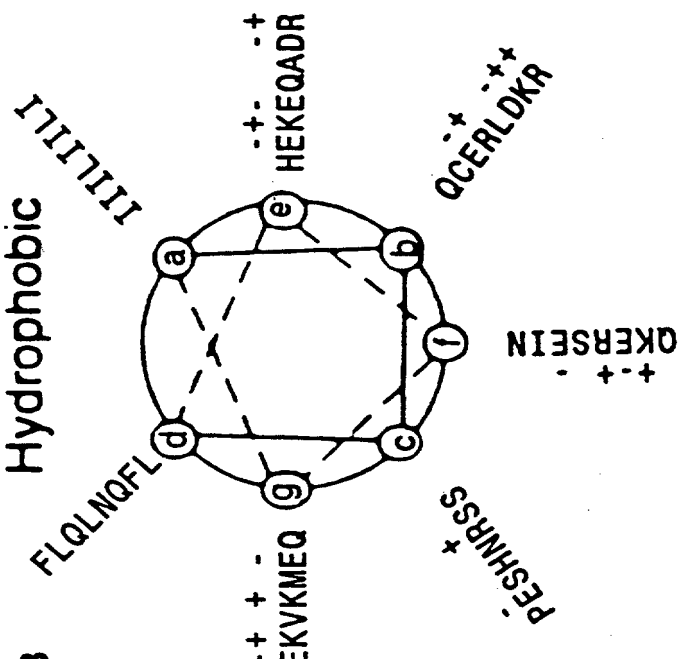


FIG. 2B

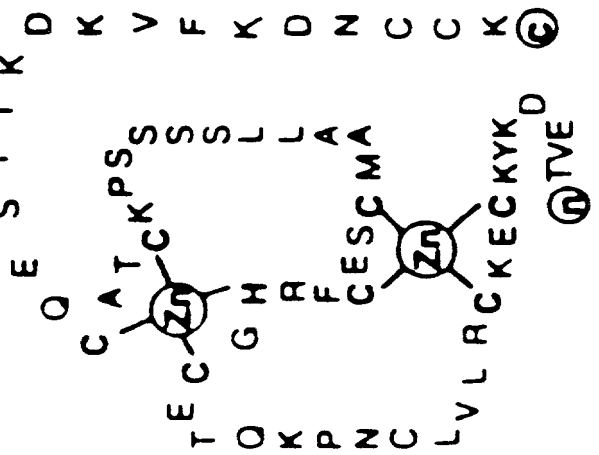


FIG. 2D

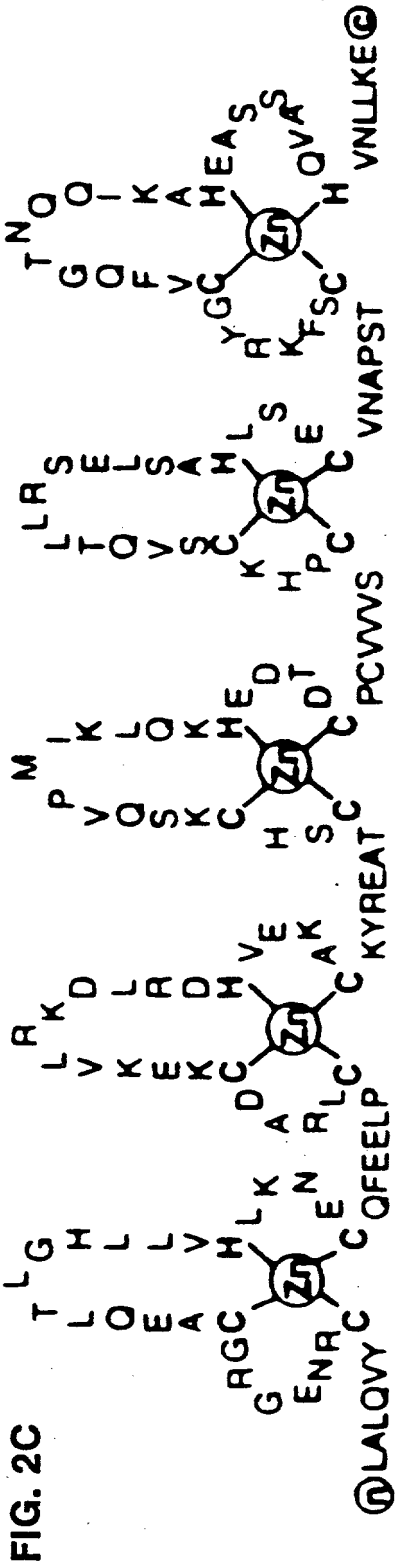


FIG. 2C

FIG. 3

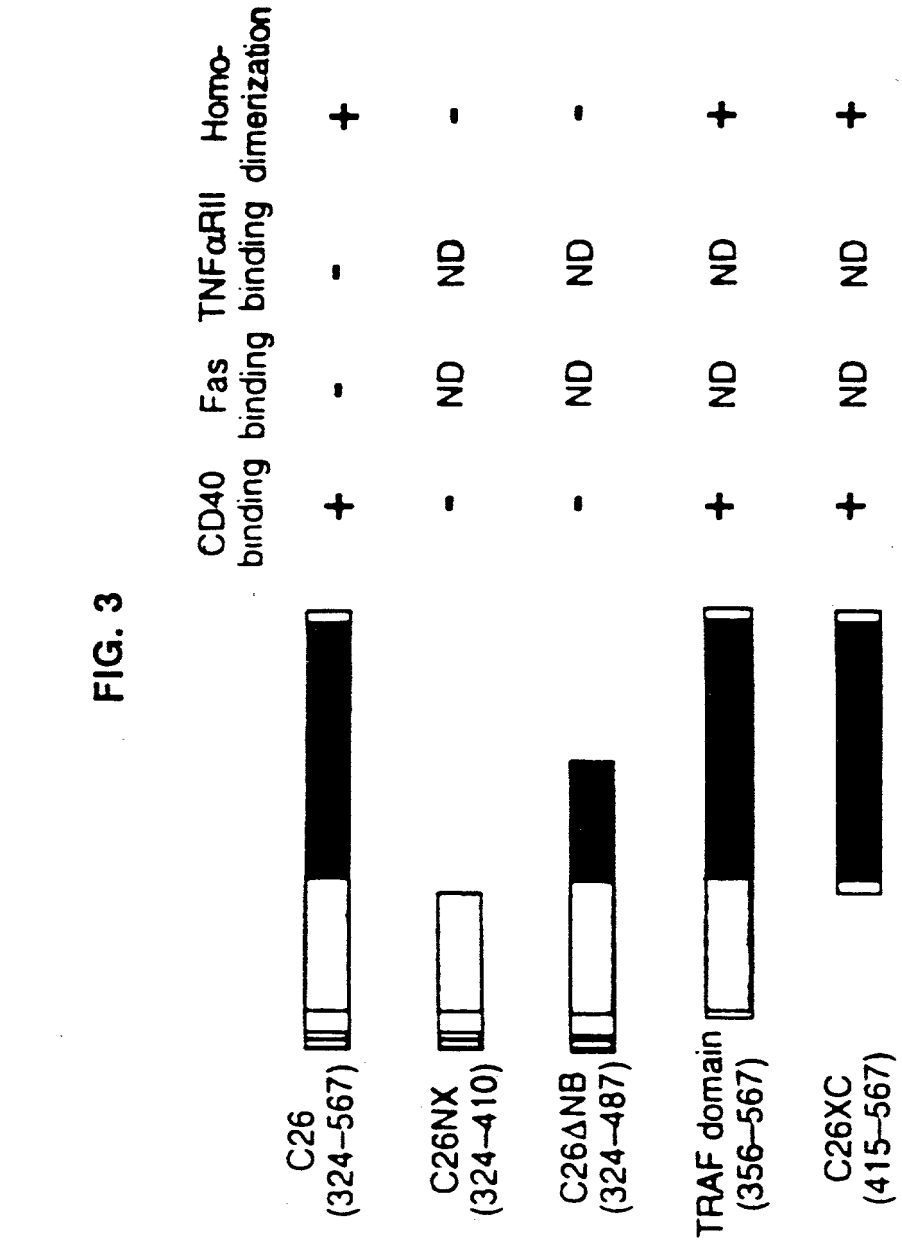
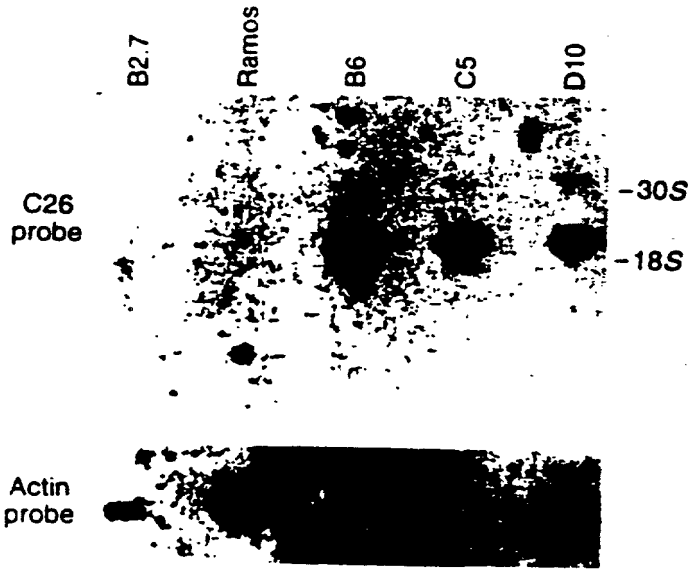

CD40 binding
Fas binding
TNFαRII binding
Homo-dimerization
C26 (324–567)
+
-
-
+
C26NX (324–410)
-
ND
ND
-
C26ΔNB (324–487)
-
ND
ND
-
TRAF domain (356–567)
+
ND
ND
+
C26XC (415–567)
+
ND
ND
+

FIG. 4A



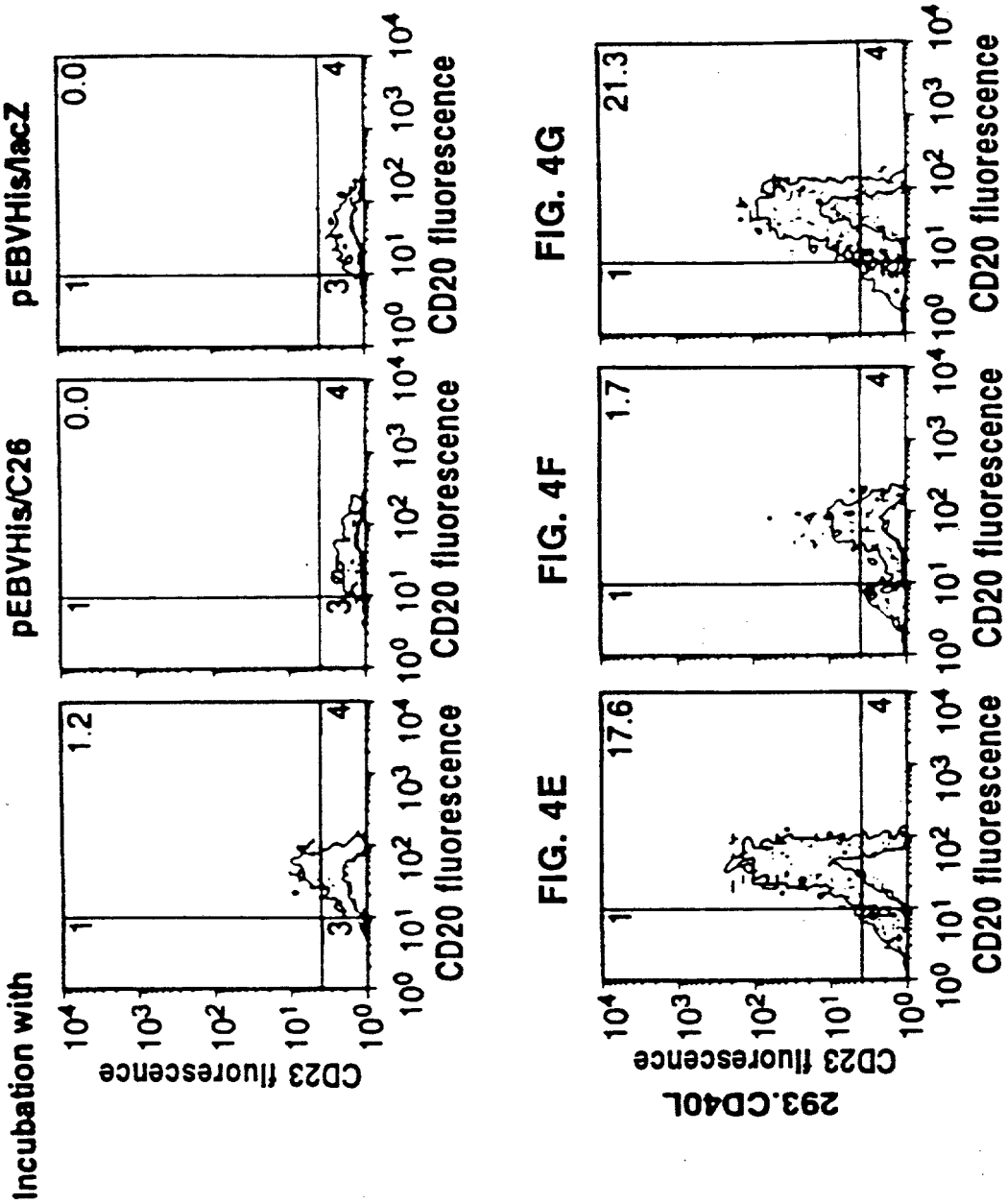


FIG. 4B
FIG. 4C
FIG. 4D
Ramos transfected with
Incubation with
pEBVHis/C26
pEBVHis/lacZ
1.2
0.0
0.0
CD23 fluorescence
CD20 fluorescence
FIG. 4E
FIG. 4F
FIG. 4G
293.CD40L
17.6
1.7
21.3

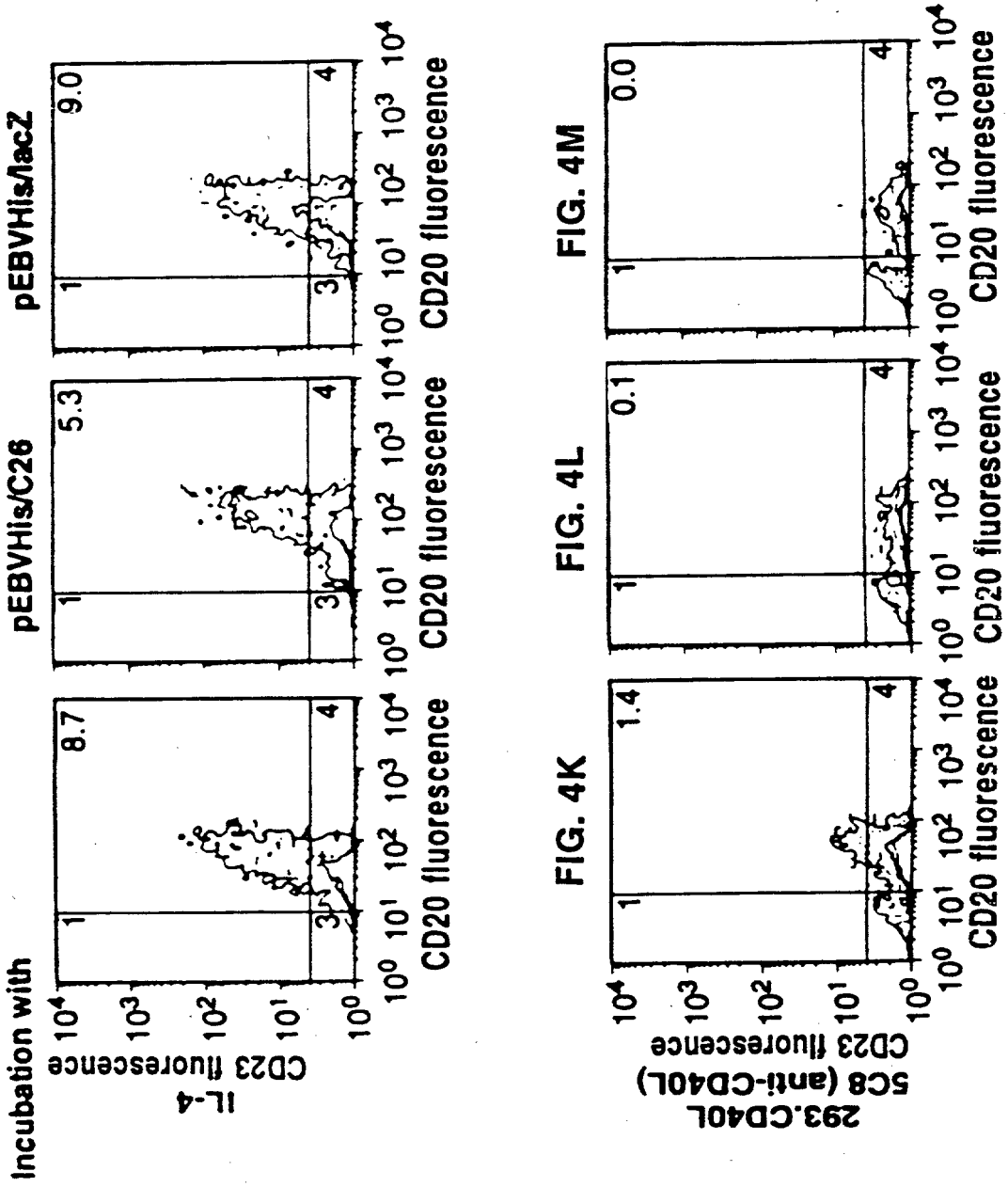


FIG. 4H
FIG. 4I
FIG. 4J
Ramos transfected with
pEBVHis/C26
pEBVHis/lacZ
Incubation with
IL-4
8.7
5.3
9.0
FIG. 4K
FIG. 4L
FIG. 4M
293.CD40L
5C8 (anti-CD40L)
1.4
0.1
0.0
CD23 fluorescence
CD20 fluorescence

FIG. 5A

```
   1 GGCGGCGGAG GATGCGCGCG GCGCCTGAGC CGGCCGAACG GGCGGCCTCG GGGTACAGGG
  61 TCCCCATTAC TTGAAGGATA AGGCTGGCAC GGCTCCGACG TCTGTGTGGA AGCTTCTCCC
 121 TCCCTTCTGA GCTTCTCTAG ACTCCTTACA GCGCACGGCA CAGAATTTCA GTTTCCTAAG
 181 ATGGAGTCAA GCAAAAAGAT GGATGCTGCT GGCACACTGC AGCCTAACCC ACCCCTAAAG
 241 CTGCAGCCTG ATCGCGGCGC AGGGTCCGTG CTCGTGCCGG AGCAAGGAGG CTACAAGGAG
 301 AAGTTTGTGA AGACGGTGGA AGACAAGTAC AAGTGCGAGA AGTGCCGCCT GGTGCTGTGC
 361 AACCCGAAGC AGACGGAGTG TGGCCACCGG TTCTGCGAGA GCTGCATGGC CGCCCTGCTG
 421 AGCTCCTCCA GTCCAAAATG CACAGCGTGC CAAGAAAGCA TCATCAAAGA CAAGGTGTTT
 481 AAGGATAATT GCTGCAAGAG AGAGATTCTG GCCCTTCAGG TCTACTGTCG GAATGAAGGC
 541 AGAGGTTGTG CGGAGCAGCT GACTCTGGGA CATCTGCTGG TGCACCTAAA AAATGAATGT
 601 CAGTTTGAGG AACTTCCCTG TCTGCGTGCC GACTGCAAAG AAAAAGTACT GAGAAAAGAC
 661 TTGCGGGATC ACGTGGAAAA GGCCTGTAAA TACCGCGAGG CCACGTGCAG TCACTGCAAG
 721 AGCCAAGTGC CCATGATCAA ACTGCAGAAA CATGAAGACA CAGATTGTCC CTGTGTGGTG
 781 GTATCCTGCC CTCACAAGTG CAGCGTTCAG ACTCTTCTAA GGAGTGAGTT GAGTGCACAC
 841 TTGTCCGAGT GTGTCAATGC CCCCAGCACC TGTAGTTTTA AGCGCTATGG CTGCGTTTTT
 901 CAGGGTACAA ACCAGCAGAT CAAGGCCCAT GAGGCCAGCT CCGCGGTACA GCACGTGAAC
 961 CTGCTGAAGG AGTGGAGCAA CTCCCTGGAG AAGAAGGTTT CCCTGCTGCA GAATGAAAGT
1021 GTTGAGAAAA ACAAGAGCAT CCAAAGCCTG CACAACCAGA TCTGCAGCTT TGAGATCGAG
1081 ATTGAGAGGC AGAAGGAGAT GCTCCGAAAC AACGAGTCCA AGATCCTTCA CCTGCAGCGG
1141 GTAATCGACA GCCAAGCAGA GAAACTGAAA GAACTGGACA AGGAGATCCG TCCCTTCCGG
1201 CAGAACTGGG AGGAAGCGGA CAGCATGAAG AGCAGTGTGG AGTCCCTCCA GAACCGAGTG
1261 ACTGAGCTGG AGAGCGTAGA CAAAAGTGCG GGGCAGGCGG CTCGCAACAC AGGCTTGCTG
1321 GAGTCCCAGC TGAGCCGGCA TGACCAGACG TTGAGTGTTC ATGACATCCG CTTGGCCGAC
1381 ATGGACCTGC GGTTCCAGGT CCTCGAGACC GCCAGCTACA ACGGGGTGCT GATCTGGAAG
1441 ATCCGTGACT ACAAGCGCCG GAAGCAGGAG GCCGTCATGG GGAAGACCCT GTCTCTCTAC
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FIG. 5B

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1501 AGCCAGCCTT TCTACACAGG TTATTTTGGC TATAAGATGT GTGCCAGGGT CTACCTGAAT
1561 GGGGACGGAA TGGGGAAAGG GACACACTTG TCGCTGTTTT TTGTCATTAT GCGTGGAGAA
1621 TATGATGCTC TGTTGCCATG GCCGTTCAAG CAGAAAGTGA CACTTATGCT GATGGATCAG
1681 GGGTCCTCTC GCCGTCATCT GGGAGATGCG TTCAAGCCTG ACCCCAACAG CAGCAGCTTC
1741 AAGAAACCCA CCGGAGAGAT GAATATCGCC TCTGGCTGCC CAGTCTTTGT CGCCCAAACT
1801 GTTCTAGAGA ACGGGACGTA TATTAAAGAT GATACAATCT TTATTAAGGT CATAGTGGAT
1861 ACCTCGGATC TGCCTGACCC CTGACAAGAA AGCAGGGCGG TGGATTCAGC AGAAGGTAAC
1921 TCCTCTGGGG GGGTGAGCTA GTGTCTTCAC GGAGGTCCTC GCCCTCAGAA AGGACCTTGT
1981 GGCGCAGAGG AAGCAGCCGG AGGAGGAGAA GGAGGTCGAG TGGCTGGCAG GAGAGCCACA
2041 TGTGAAAACA GACCCCAACG GATTTTCTAA TAAACTAGCC ACACCCACTC TGAAGGATTA
2101 TTTATCCATC AACAAGATAA ATACTGCTGT CAGAGAAGGT TTTCATTTTC ATTTTAAAAG
2161 ATCTAGTATT AAGGTGGGAA CATATATGCT AAAAAGAAAC ATGATTTTTC TTCCTTAACT
2221 TAAACACCAA AAAGAGAACA CATGTGGGGG TAGCTGGAGT GTGTACAGTA CCTCGAGGGC
2281 TTAAAATCAT AAACAATCAC ATACTCATCC TAAAATTCAG GGTGCAACTC CGTTTCAAAT
2341 ATTGTATATT GTCTATTTA
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FIG. 6A

1	CGGGGGAGCG	CGGCGCGGCC	GCCGCGTGCG	CGAGCCGGGG	TTGCAGCCCA	GCCGGGACTT
61	TCCAGCCGGC	GGCAGCCGCG	GCGGTCGTCG	GCTCTTCCCC	GCCCCCCGTC	ATGGGGCAGC
121	CCGGGGAGCA	GAACGCTGCG	GACCGCGGCG	GAGGACGCGC	CCGGCGCCCC	TGAGCCGGCC
181	GAGCGGCGAC	GGACCGCGAG	AACTCCTCTT	TCCTAAAATG	GAGTCGAGTA	AAAAGATGGA
241	CTCTCCTGGC	GCCCTGCAGA	CTAACCCGCC	GCTAAAGCTG	CACACTGACC	GTAGTGCTGG
301	GACGCCAGTT	TTTGTCCCTG	AACAAGGAGG	TTACAAGGAA	AAGTTTGTGA	AGACCGTGGA
361	GGACAAGTAC	AAGTGTGAGA	AGTGCCACCT	GGTGCTGTGC	AGCCCGAAGC	AGACCGAGTG
421	TGGGCACCGC	TTCTGCGAGA	GCTGCATGGC	GGCCCTGCTG	AGCTCTTCAA	GTCCAAAATG
481	TACAGCGTGT	CAAGAGAGCA	TCGTTAAAGA	TAAGGTGTTT	AAGGATAATT	GCTGCAAGAG
541	AGAAATTCTG	GCTCTTCAGA	TCTATTGTCG	GAATGAAAGC	AGAGGTTGTG	CAGAGCAGTT
601	AACGCTGGGA	CATCTGCTGG	TGCATTTAAA	AAATGATTGC	CATTTTGAAG	AACTTCCATG
661	TGTGCGTCCT	GACTGCAAAG	AAAAGGTCTT	GAGGAAAGAC	CTGCGAGACC	ACGTGGAGAA
721	GGCGTGTAAA	TACCGGGAAG	CCACATGCAG	CCACTGCAAG	AGTCAGGTTC	CGATGATCGC
781	GCTGCAGAAA	CACGAAGACA	CCGACTGTCC	CTGCGTGGTG	GTGTCCTGCC	CTCACAAGTG
841	CAGCGTCCAG	ACTCTCCTGA	GGAGCGAGTT	GAGTGCACAC	TTGTCAGAGT	GTGTCAATGC
901	CCCCAGCACC	TGTAGTTTTA	AGCGCTATGG	CTGCGTTTTT	CAGGGGACAA	ACCAGCAGAT
961	CAAGGCCCAC	GAGGCCAGCT	CCGCCGTGCA	GCACGTCAAC	CTGCTGAAGG	AGTGGAGCAA
1021	CTCGCTCGAA	AAGAAGGTTT	CCTTGTTGCA	GAATGAAAGT	GTAGAAAAAA	ACAAGAGCAT
1081	ACAAAGTTTG	CACAATCAGA	TATGTAGCTT	TGAAATTGAA	ATTGAGAGAC	AAAAGGAAAT
1141	GCTTCGAAAT	AATGAATCCA	AAATCCTTCA	TTTACAGCGA	GTGATCGACA	GCCAAGCAGA
1201	GAAACTGAAG	GAGCTTGACA	AGGAGATCCG	GCCCTTCCGG	CAGAACTGGG	AGGAAGCAGA
1261	CAGCATGAAG	AGCAGCGTGG	AGTCCCTCCA	GAACCGCGTG	ACCGAGCTGG	AGAGCGTGGA
1321	CAAGAGTGCG	GGGCAAGTGG	CTCGGAACAC	AGGCCTGCTG	GAGTCCCAGC	TGAGCCGGCA
1381	TGACCAGATG	CTGAGTGTGC	ACGACATCCG	CCTAGCCGAC	ATGGACCTGC	GCTTCCAGGT
1441	CCTGGAGACC	GCCAGCTACA	ATGGAGTGCT	CATCTGGAAG	ATTCGCGACT	ACAAGCGGCG

FIG. 6B

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1501 GAAGCAGGAG GCCGTCATGG GGAAGACCCT GTCCCTTTAC AGCCAGCCTT TCTACACTGG
1561 TTACTTTGGT TATAAGATGT GTGCCAGGGT CTACCTGAAC GGGGACGGGA TGGGGAAGGG
1621 GACGCACTTG TCGCTGTTTT TTGTCATCAT GCGTGGAGAA TATGATGCCC TGCTTCCTTG
1681 GCCGTTTAAG CAGAAAGTGA CACTCATGCT GATGGATCAG GGGTCCTCTC GACGTCATTT
1741 GGGAGATGCA TTCAAGCCCG ACCCCAACAG CAGCAGCTTC AAGAAGCCCA CTGGAGAGAT
1801 GAATATCGCC TCTGGCTGCC CAGTCTTTGT GGCCCAAACT GTTCTAGAAA ATGGGACATA
1861 TATTAAAGAT GATACAATTT TTATTAAAGT CATAGTGGAT ACTTCGGATC TGCCCGATCC
1921 CTGATAAGTA GCTGGGGAGG TGGATTTAGC AGAAGGCAAC TCCTCTGGGG GATTTGAACC
1981 GGTCTGTCTT CACTGAGGTC CTCGCGCTCA GAAAAGGACC TTGTGAGACG GAGGAAGCGG
2041 CAGAAGGCGG ACGCGTGCCG GCGGGAGGAG CCACGCGTGA GCACACCTGA CACGTTTTAT
2101 AATAGACTAG CCACACTTCA CTCTGAAGAA TTATTTATCC TTCAACAAGA TAAATATTGC
2161 TGTCAGAGAA GGTTTTCATT TTCATTTTTA AAGATCTAGT TAATTAAGGT GGAAAACATA
2221 TATGCTAAAC AAAAGAAACA TGATTTTTCT TCCTTAAACT TGAACACCAA AAAAACACAC
2281 ACACACACAC ACGTGGGGAT AGCTGGACAT GTCAGCATGT TAAGTAAAAG GAGAATTTAT
2341 GAAATAGTAA TGCAATTCTG ATATCTTCTT TCTAAAATTC AAGAGTGCAA TTTTGTTTCA
2401 AATACAGTAT ATTGTCTATT TTTAAGGCCT CCAAAAAAAA AAAAAATTCC GGCCG
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